Immunity and Hypersensitivity Relationship to Disease in Man

Report of the Ninth M & R PEDIATRIC RESEARCH CONFERENCE

Issued by M & R Laboratories, Columbus 16 Ohio

The purpose ot these conferences is to assist in the correlation of the latest research information on topics of general interest in the practice of pediatrics, and to stimulate further research by the exchange of information. These objectives coincide with the in tention f the scientific staff of M & R Laboratories, manufacturer f Similac® to keep informed of current developments in pediatric research. It has become pparent that a large audience is interested in the conferences and f r this reason M & R Laboratories is publishing this report. Previous conferences were reported under the t tles listed bel w

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The Ninth M & R Pediatric Research Conference, a symposium on immunity and hypersensitivity relationship to disease in man, was held under the auspices of the Department of Pediatrics of Stanford University School of Medicine, at Lane Hall, San Francisco, California, on October 30 and 31 1953.

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Introduction

DR. SIDNEY RAFFEL. Effective studies of the nature of immunologic phenomena began to blossom for the second time some fifteen years ago after such various disciplines as physical chemistry, nuclear physics, biochemistry, pharmacology, and endocrinology had provided the ultracentrifuge, electrophoretic apparatus, tagged atoms, purified endocrine secretions and specific cellular poisons. Many concepts previously not studied for lack of suitable methods were explored through these new techniques. Thus contributions from the physical sciences applied to the biological and clinical sciences have produced progress in the understanding of immunology.

Immunologic investigations follow two main paths of interest. The first is concerned with infectious disease and the second with studies of hypersensitivity and of immunologic disease, i.e., disease predicated upon the destruction of an individual's cells by the action of antibodies. Both areas of interest are closely related because the same considerations of specificity, of antigenicity and of host response apply in acquired immunity to parasites and toxins, in antibody formation by the hypersensitive subject, and in the manufacture of autoantibodies against tissue components, or the acquisition of antibodies transplacentally or by transfusion. Knowledge of such phenomena acquired during the past ten or fifteen years constitutes the basis for the present symposium.

The immunologic response of the host has been found to differ qualitatively as well as quantitatively depending upon the chemical nature of an antigen, the route by which this gains access to the tissues, the intensity and duration of exposure of the tissues to the antigen, and the species of the responding animal. Thus administration of rabbit serum albumin to the horse results in antibody with an electrophoretic mobility between beta and gamma globulin; this antibody will not flocculate with antigen except in a very narrow range of mutual concentrations. On the other hand, rabbit

serum globulin administered to a horse evokes the formation of anti body of high molecular weight, about 900,000, with a very broad reactive ability with its antigen. Analagous information is avail able for various antibody responses in the human being.

Studies of infectious disease have also clarified our under standing of the natures of immunizing substances. It was once thought that acquired resistance to all bacteria might depend upon antigenic components of the capsule or cell surface. The fallacy of such a generalization is apparent from the example of anthrax bacillus, for antibodies formed against the highly antigenic capsular substance of this bacterium do not confer immunity The development of antibodies by the host in this case reflects simply a con f rmity to the physiologic law that the animal body responds in this way to substances foreign to the tissues. There is not inherent this response a protective function.

I the field f diseases f hypersensitivity an awareness of the abil'ties of simple chemical substances to become antigenic through association with tissue protein has increased our understand g of the occurrence of these affections. Tissue changes which take place in experimental animals undergoing hypersensitive reactions ha e been compared with alterations observed in human subjects in some disease states, and this comparison serves as a basis for the classi fi tion f rheumatic fever as a disease of hypersensitivity

Diseases of immunologic origin such as erythroblastosis fetalis and certain instances f hemolytic anemia and thrombocytopenia purpura define circumstances in which the maternal organism or the affected subject himself seems bent upon fetal or self-destruction

During the course of this ymposium several facets f this complex subject of immunity and hypersensitivity will be more fully expl red.

Cellular Sources of Antibodies

Nature of antibody

DR. WILLIAM E. EHRICH Since the turn of the century the globulin nature of antibody has been recognized. Recently it has been shown by the electroph retic technique that antibody migrates with or close t gamma globulin, although not all gamma globulin is antibody The gamma gl bulin or proteins similar to gamma globulin produced f cases f multiple myeloma are non antibody protein. Hyperimmunization of rabbits leads to the replacement of

all non-antibody globulin by antibody globulin and, in contrast, patients with congenital agammaglobulinemia lack the ability to form antibody. In a discussion of the cellular sources of antibody non-antibody gamma globulin must be considered.

The plasma cell as source

The cells commonly considered as sources of antibody and gamma globulin are the reticuloendothelial cells, the lymphocytes, and the plasma cells. Our current studies point to the plasma cell as the source of antibody and gamma globulin. Other investigators favor the lymphocyte, or classify the plasma cell as a functional stage of reticuloendothelial cell or lymphocyte development.

Reticuloendothelial cells or macrophages are phagocytic and arise from nonphagocytic undifferentiated mesenchymal cells. The macrophage may occur either as a fixed reticuloendothelial cell, a free monocyte in the blood and lymph, or a histiocyte in tissue. A fixed reticuloendothelial cell develops from a fixed mesenchymal cell. Becoming migratory in tissue it is classified a histiocyte. Circulating monocytes arise from fixed undifferentiated mesenchymal cells passing through a blast form. The monocyte migrating into tissue, or grown in tissue culture may develop into a histiocyte undistinguishable from those derived from fixed reticuloendothelial cells.

The view that mature macrophages or lymphocytes can change into plasma cells is at variance with our knowledge of ontogenesis. Lymphocytes and plasma cells arise from undifferentiated mesenchymal cells passing through blast forms. Lymphocytes develop from lymph blasts and are small pyknotic cells which seem to disintegrate within a few days, in contrast to the macrophage, and are found reduced in number in the circulating blood following the release or administration of adrenal cortical steroids. Plasmoblasts undergo differentiation and maturation, to disintegrate within one week.

Clinical evidence

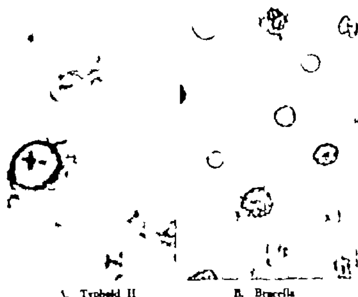
On clinical grounds there is no support for the concept that either antibody or gamma globulin arises from the lymphocyte or reticuloendothelial cell. Hyperglobulinemia is an unusual finding in cases of lymphocytic or monocytic leukemia or of reticuloendothelial cell sarcoma. Experimentally no one has isolated antibody from the lymphocytes of the thymus cortex or the circulating blood.

Macrophages harvested from the peritoneal cavity do not yield antibody

These results contrast sharply with the clinical and experimental observations made on plasma cells. With plasma cell proliferation in patient, experimental animal, r tissue culture, a rise in antibody formation or non-antibody gamma globulin is noted. The highest levels of serum gamma globulin are to be found in patients with multiple myeloma

Cytologic evidence

Cytologically the plasma cell, in contrast to the reticuloendothelial cell or the lymphocyte resembles a cell actively engaged



A. Typhoid H B. Brucella

Figure 1 Agglutination of bacteria on the cell membrane of plasma cell, 1000. Reprinted from the Proceedings of the Society of Experimental Biology and Medicine 74 731 (1950)

in protein synthesis. The cytoplasm is rich in ribonucleic acid and the electronmicroscope reveals structures found only in cells actively elaborating protein. Russel bodies, believed to represent retained protein secretions, re frequently found in plasma cells With phase microscopy dark droplets believed t be organs of protein synthesis, if not synthesized protein, have been described within the

cytoplasm f the plasma cell. These droplets are not found in the lymphocyte or reticuloendothelial cell.

Immunologic evidence

Immunologi evidence is available in support of the concept that the plasma cell elaborates antibody Plasma cells isolated from an antibody f rming lymph node will agglutinate bacteria and dissolved antigen on the cell membrane. Macrophages o lymphocytes do not manifest this activity (figure 1) A globulin has been successfully extracted from plasma cells isolated from a case f multipl myeloma, having the same electrophoretic m bility as the serum globulin producing the hyperglobulinemia f the patient.

These observations lend support to a genetic theory of anti body f rmation, by which the antigen does not act on gamma gl bulin, as suggested by Pauling, or on the ytoplasm of the anti body forming cells, as postulated by Burnett, but rathe by which antigen functi ns as an organizer inducing undifferentiated mesen chymal cells the plasmablast to f rm a specifi plasma cell equipped to synthesize a specific antibody directed against the antigen responsible f r its creation.

Viral Antigens and Antibodies

DR. A. W DOWNIE The characterization f virus antigens i extremely complex and dependent in part on the interaction f virus antigen with antibody The antigenic structures f vaccinia and influenza viruses ha e been th most extensi ely studied ystems t date.

Vaccinia antigens

The accinia virus elaborates at least three specifi antigeni substances, (1) soluble antigen p tein in nature, with a mol ular weight f 240 000, (2) ucleop otein antigen, and (3) hemagglutinin. The presence f a neutralizing antibody bserved in animals given the active virus has raised the question f the relationship of these known antigens t the immunizing property of the active virus particle.

The solubl antigen possesses both heat stable and heat labile components, producing tw distinct antibodies that react with single antigen called the LS antigen

The ucleoprotein antigen can be prepared by alkaline

traction f the virus, and probably constitutes at least half f the virus particle. The injection into rabbits of noninfective alkaline extracts of virus particles produces antibodies.

The hemagglutinin f vaccinia, antigen cally distinct from LS antigen and nucleoprotein antigen, is probably lipoprotein in na ture. It is associated with a particle some 65 mμ in size.

While each of these antigens separately induces antibody f mation in the rabbit, the antibodies d not neutralize the active virus n confer immunity to the animals. Serum obtained from a rabbit immunized with active virus to which has been added the three separated antigens, thereby absorbing out specific antibodies, still possesses its protective or neutralizing antibody with titer unimpaired. The immunizing property of the active virus particle is not found in its component parts as known today and only infection r hyperimmunization with a live vaccinia virus will induce a high degree f immunity with the production of neutralizing anti body

Influenza antigen

The influ nza virus can be btained in a relatively pure state because it is readily absorbed by the red blood cells f many species. However even the most highly purified preparations of influenza virus still co tain antigens characteristic of the host from which the preparation is derived. Chick embryo isolates contain antigens characteristic of llantoic fluid, and mouse lung isolates contain antigens characteristic f normal mouse lung

Antisera to the influenza virus will neutralize the virus, inhibit hemaggl tination, and fix complement in the presence f the virus. Particles of smaller size than the virus have been prepared which have hemagglutinating properties.

Two antigens may be shown to fix complement. A soluble antigen is derived from infected tissue that is type-specific but not strain-specific, i.e., the soluble antigen of a type A strain will not fix complement with a type B antiserum, but will react with antisera t serologically different strains within type A. The second complement fixing antigen is associated with the virus particle and is strain-specific. Immunity to infection with influenza A viruses is largely strain-specific and the hemagglutinin-inhibiting activity of an antiserum closely parallels neutralizing activity

Factors determining immunity

Immunity subsequent to recovery from virus infections is de-

pendent on the production and persistance of the specific antibody. Nonspecific factors may determine some degree of resistance and offer an explanation for the variation in resistance shown by a population of nonimmune individuals. Nonspecific virus-inhibiting properties of sera and tissue extracts seem to be associated with mucoproteins.

The presence and amount of specific antibody will not necessarily determine the degree of immunity to a virus infection under natural conditions of exposure.

The nature of the virus itself determines in some measure the immune response. Low immunity appears in some instances to be associated with a persistance of the virus in the tissues and an associated allergic reactivity to the inoculated virus. This situation gives rise to a chronic and sometimes relapsing type of infection.

Clinical influenza may occur in a person possessing antibody against the infecting strain. The influenza virus can invade the mucosa of the upper respiratory tract without gaining entry into the bloodstream. Immunity under these circumstances is dependent on the concentration of antibody at the point of entry (in the secretions of the respiratory mucosa). In generalized infections where viremia must occur serum antibody is more effective. The long duration of immunity following such virus diseases as yellow fever and the exanthemata has provoked much discussion. It has been suggested that the virus or virus antigen may persist in the tissue to provide a stimulus for further antibody formation. The psittacosis virus in birds, the lymphocytic choriomeningitis virus in mice, and the herpes simplex virus in man persist in tissues and remain latent until external circumstances upset the host-parasite relationship to reveal the presence of the virus. As yet we have no epidemiologic, clinical, or experimental evidence to support the concept that the viruses of smallpox, yellow fever or childhood exanthemata persist in the tissues.

The Characterization and Production of Antibody Protein

DR. DAN H. CAMPBELL. The clinical investigator as well as the biochemist should have some working knowledge of the nature of antibody that is involved in the antigen-antibody reaction.

Size and shape of antibody

The molecular size of an antibody is an extremely important

consideration, for it has bearing on such phenomena as diffusion f antibody across the placental barrier as well as the blood-brain barrier and permits estimation of the number of molecules involved in skin reactions, hem lysis, or agglutination reactions. With the excepti n of an antibody found in the serum f horses sensitized with pneumococcus polysaccharide the molecular weight of anti body is about 160,000 Antibody is a cigar-shaped structure mea suring 250 Å i length and 35 Å in diameter

The electrophoretic properties of the antibody molecules that confer immunity and produce the usual serological reactions of flocculation and precipitation resemble those of gamma gl bulin. Antibodies not giving the usual serological reactions (e.g. reagin of allergic serum or the blocking antibody of anti Rh serum) migrate in an electrophoretic fi ld with globulins of the α_2 or β variety

Solubility and chemical composition

In experimental studies, the solubility of antibodies assumes importance. Generally the water-insoluble component increases during the immunization of rabbits and is reflected in great amount of antibody in the water insoluble portion of gamma globulin.

The chemical composition f antibody protein does not dis-tinguish t from serum proteins nor permit differentiation between antibodies produced by differing antigens. Carbohydrat and lipid groups are integral parts of antibody molecules, with the lipid groups influencing only the rate of antigen antibody interaction, and not the primary combinations that may occur End-group analyses of terminal amino acids in rabbit antibody indicates a sequence f amino acids imilar to that in normal gamma globulin—alanine, leucine, valine, aspartic and probably glutamic acid.

When an animal is injected with an antigen even as molecularly pure as crystalline ovalbumin the resulting antiserum con *tains antibody molecules differing widely in their immunological* and physical properties. This heterogenicity of antibody is important in considering the reaction capacity and potentialities of serum, for the titer of an antiserum is the result f competing reactions by a heterogeneous population of antibody molecules.

Combining sites

Antibody-combining sites have been the subject f much study and speculation. Those antibody molecules involved in the f rma-

tion f insoluble complexes are considered to have two combining sites. This finding has led t the concept that nonprecipitating anti bodies theref re have nly one combining site however no direct evidence for this is yet vailable. The relative positions f the com bining sites are unknown. Removal of one half the diphtheria anti t xin molecule by pepsin digestion leaves all the riginal combining sites with the remaining half. It is concei abl that the number f ites may be the same in all antibody molecules but that the placement aries. If the sites are close together nly singl antigen molecules can react, spatial considerati ns excluding other reactions. Th antibody-combining site is relatively small, with an area f 500-700 Å hence metalloorganic complexes or ingl at ms such as iodine may act as pecific determinants. While the combining site has highly specific complementary structure relati t antigenic determinants, the site does not influence the antigenic specificity f the molecules.

Factors influencing antibody formation

Speculation on antibody f rmation must acknowledge many factors. Some f the more important are (1) animal species—data obtained on ats are not entirely comparable to data obtained on rabbits or humans (2) the chemical and physical nature f the antigen employed—in rder to produce antibodies the material must be digestible *in vivo* (3) the age and past history of the animal—the antibody f rming mechanism f young animals is limited and an occasional good response in test animal may reflect pri exposure to the antigen substance containing trace f the component under study (4) the type f test t be used to determine the presence and amount f antibody is important since some are more sensitive than thers. If an excess of inhibiting antibody is present, high concentration f circulating antibody giving a precipitation or agglutination reaction may be missed.

When an an mal is immunized, very little change occurs in the physical properties f the serum. With continuing immunization an increase in gamma gl bulin and sometimes beta globulin occurs. The electrophoretic patterns f three pools f rabbit serum re show in figure 2. The highest tit serum has the greatest amount f gamma glob lin with two distinct peaks With strenuous immunization the concentration f gamma gl bulin, which n rmally constitutes 15 percent f the total serum protein, may rise t tw three times this lue, with antibody constituting about half f the total gamma globulin. While the question f maximum anti-

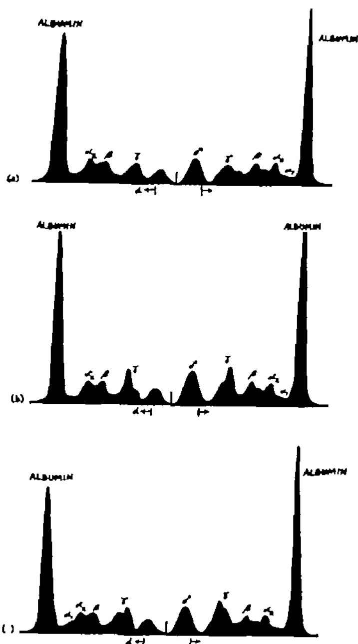


Figure 2 Electrophoretic patterns f serum from rabbits immunized against arsanilic-azo-bovine globulin (RB$_0$) showing differences in the gamma globulin component. = 1 mg. of anti-R, b = 4 mg. f anti-R and = 5 mg. f anti-R per ml. f serum. Reprinted from The Journal of the American Chemical Society 73:4611 (1951)

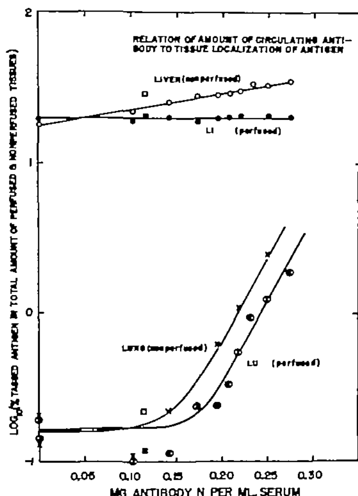


Figure 3. The relation of circulating precipitating antibody (antiserl-famile-ans-hemocyanin) to localization f homologous S^{35} labeled antigen. Reprinted from the Journal f Immunology 72 131 (1954)

body f rming capacity f the body has not been studied, it must be assumed, if there is maximum, that simultaneous immunization *with multiple antigens will produce less antibody for gi en antigen* than will an antigen injected alone.

The increase in gamma globulin after immunization cannot be entirely accounted f as antibody by serological testing hence it

is referred to as normal gamma globulin. Many immunologists, however consider all gamma gl bulin as antibody formed against some unkn wn antigen. When potent antigens are used, antibody f rmation can be very rap d. The injection of C labeled leucine into rabbits immunized against ovalbumin results in the appearance of circulating radioactive antibodies in 24 to 48 hours. Once anti body is formed, the injection of additional specific antigen is f l lowed by a rapid rate of antibody formation. The localization f antibody t tissues or organs is not influenced by irculating anti body until the concentration of antibody app oaches 0.1 mg. per ml of serum (figure 3)

Theories of antibody formation

The two main theories of antibody formation remain in about the same state as they were ten years ago One concept is that antigen directly shapes the gamma globulin m lecule, either by simple mechanical molding or by changing the sequence of amino acids and thereby imposing pecificity The other concept regards antigen as influencing cell organizers, which i turn direct the synthesis of specific proteins. The first concept involves the continuing presence of antibody while the latter does not in l antigen once the antigenic pattern has been imposed on cellular enzyme systems. Antigen retention in tissues thus assumes a very important ole. Experimental study f this problem is difficult be cause antigen quickly disappears beyond chemical detection, b t may still be present and account for contin ing antibody f rmation If antigen persists in tissues it may do so in a partially degraded form, hence must be isolated and identified as such.

Metabolism of Antibody

Dr. Frank J Dixon Since the rates of synthesis and degrad ation f antibody appear to correlate with the amount f antigen in the host, the study of antibody metabolism requires the use f an antigen which can be traced quantitatively in vivo Purified heterologous serum protein antigens labeled with I[1] would seem ideal for this purpose They are readily traced, call f rth a relatively simple immunologic response, equilibrate within the host s plasma protein pool, ha access to all tissues, and are not appreciably concentrated o retained by the latter Because they are catabolized rapidly they permit bservations on antibody metabolism during

th presence f antigen, and later in its absence. Since these anti gens d not persist f long in the host, the synthesis of antibody is not complicated by prolonged antigenic stimulation, and the degradation f antibody is not influenced by continuous anti body-antigen union in th tissues. The antibody f und in response to these antigens can be quantitated by the precipitin reaction.

Antibody anabolism

As shown in figure 4 the first indication f antibody produc tio is an acceleration in the rate at which antigen is eliminated from the circulation. Following a primary intravenous injection into

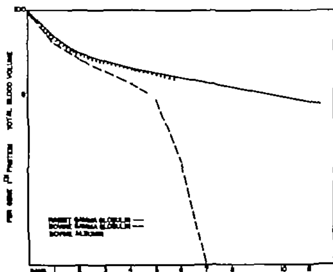


Figure 4. Disappearance of I[131] labeled proteins from the blood of rabbits. Reprinted from the Journal of Allergy 24:552 (1953)

rabbits f I labeled rabbit or bovine gamma globuli of I labeled bovine albumin, the homologous rabb t gamm globulin equilibrates with the intravascula and extravascul components f the plasma protein pool f the first 48 hours. It is subsequently lost from the blood t logarithmic rate with *half lif f five days*, the *rate t which homologous gamma* gl bulin is catabolized. The blood level f bovine albumin is imil to that f rabbit gl bulin for the first seven day after

and then diminishes at a rate comparable to that observed for antibody decline. Whether or not this basal rate of synthesis continues in the absence of antigen cannot be established with tracer techniques. It seems unlikely that heterologous serum proteins so easily catabolized would persist in the host for months.

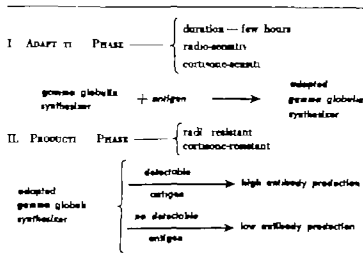


Figure 7 Phases of antibody production.

Phases of antibody synthesis

From these observations and from studies on the effects of x ray and cortisone on antibody production, the antibody response can be divided into two phases an adaptation phase and a production phase (figure 7) The adaptation phase, of short duration, probably representing an initial period of adjustment of the host to antigen, is sensitive to cortisone and x ray In rabbits the adaptation phase of *antibody production can be inhibited by 400r whole* body radiation (about one half an LD_{50} dose of radiation) 5 mg of cortisone per kg of body weight per day Radiation is most *effective if administered prior to the antigen, and once this adaptive* phase is complete, continued antibody response is relatively unaffected by radiation or cortisone. The adaptation phase probably represents some modification of the gamma globulin synthesizing mechanism under the influence of antigen, which results in the pro-

duction of altered globulin or antibody The rate of production f antibody is in part related to the quantity f detectable antigen in the host.

The metabolic characteristics f the host determine the catabolic fate of proteins as illustrated in table 1 The half lives of gamma gl bulin and albumin are similar fo given pecies. Elev ting the metaboli rate of rabbits by thyroid administration increases the rate of antibody catabolism and significantly decreases its half life.

Table 1

Half life of I labelled homologous gamma globuli and albumin

Species	No. of subjects	Method of fractionation	Gamma Globulin: Average half lif with standard deviation—days	Albumin: Average half lif with standard deviation—days
Beef	4	Alcohol	21.2 ± 1 7	20 7 ± 1 1
Human				
6 mo.—8 yrs	11	Alcohol	20.3 ± 4 1	
Adult	14	Alcohol	13 1 ± 2 8	15 3 ± 2.0
Dog	8	Alcohol	8 0 ± 1 1	8 3 ± 1.4
Monkey	10	Ammonium sulfate	6 6 ± 1 6	
Rabbit	9	Alcohol	4 6 ± 0 8	5 8 ± 1 4
	9	Ammonium sulfate	5 7 ± 1 2	
Guinea pig	13	Ammonium sulfat	5 4 ± 0 9	
Mouse	27	Ammonium sulfate	1.9	1.2

Antibody catabolism

Antibody catabolism is dependent on at least one other factor the presence or absence f specific antigen. In the absence f anti gen, antibody is catabolized t the same rate as nonspecific gamma globulin. As determined by immun logic and isotopic tracer techniques, this rate varies considerably from one species to another (tabl 1) The l wer metabolic rate, especially during the earlier months f life, may explain the sl wer rate of gamma globulin catabolism bserved in infants and children, but it is likely that other unkn wn fact rs are also determinants in this situation.

When specifi antigen is present i the host, circulating antibody and antigen combine f rming complexes which are rapidly remo ed from the irculation and catabolized. When rabbits are

given passive transfers of I^{131} labeled rabbit antibody and then challenged with specific antigen, the labeled antibody is removed from the circulation within a few hours. Following its removal fr m the circulation the antibody is rap dly catabolized as indi cated by the appearance f nonprotein-bound I in the urine. Similar studies of the fate of labeled protein antigens indicate that when antigen and antibody combine they are catabolized at com parable rates. This observation is not in keeping with the assumption that antigeni substances are not readily catabolized by the host and therefore persist and stimulate prolonged antibody syn thesis

The pattern of antibody catabolism in the p esence of antigen depends in part on the physicochemical characteristics of the tigen and its distribution within the body Antibody-antigen complexes formed and irculating within the intravascular and extravascula fluids are probably fixed and rapidly catabolized by circulating phagocytic cells. I contrast, rabbit antimouse kidney antibodies becoming fixed to the glomeruli of the mouse are lost at half life rate of 20 to 21 days. Antibody so fixed is pparently protected from cells with great proteolytic ctivity

Hormonal Influences on Antibody Mechanism

DR. THOMAS F DOUGHERTY The fact that there is so much conflicting evidence concerning the relationship of adrenal cortical h rmones t the production of antibodies is du in part t a lack of delineation of the means of measuring the process f antibody production. At least three aspects of this process should be considered. Like other proteins, antibody must be synthesized in cells and, once f rmed, must be released to body fluids, eventually to be utilized, i. e. catabolized.

Effect on circulating antibody

It has been reported that administration f adrenal cortical hormones, stimulation of endogenous secretion of these hormones by administration f ACTH, may produce an increase of circulating ntibody In the first description of this effect, t was noted that the increase was f very brief duration and was variabl in the xtent to which it occurred. Since the presence of antibody had been

demonstrated in lymphocytes and since lymphocytes are destroyed following administration of or an increase in blood levels of adrenocortical hormones from endogenous sources, it was suggested that lymphocytolysis is accompanied by a release of antibody from this preformed source, although without implication that the adrenal cortex is essential to the synthesis of antibody. It was also pointed out that, although adrenal cortical hormones enhance the destruction of lymphocytes, antibody did not appear in adrenalectomized animals, and that therefore the mechanism of release of antibody was not dependent entirely upon the presence of the adrenal cortex. These early results have not been duplicated, although some investigators have confirmed the nonspecific anamnestic response induced by adrenal cortical secretions.

Evidence concerning the effect of the removal of the adrenal on the rate of production of antibody is conflicting. Several investigators have found that removal of the adrenals enhances antibody synthesis, others have reported that the formation, particularly of hemolysins, is definitely impaired in the adrenalectomized rat, and still others have reported that adrenalectomy is without effect upon the antibody forming mechanism. The administration of adrenal extracts for a prolonged period of time has been reported to increase the level of antibody, while ACTH and cortisone, given for any prolonged period of time, have been demonstrated to depress antibody formation. In man, particularly, administration of these hormones is without any effect on antibody titer.

In a study done by Merick, the formation of precipitating antibody to pneumococcal polysaccharides after administration of ACTH (17 cases) or of cortisone (12 cases) was not depressed, but actually slightly increased compared to three control subjects. Little effect of these hormones on production of agglutinins to typhoid bacilli was found, although it should be noted that isohemagglutinins were increased four-fold in 4 of 11 hormone-treated patients. Serum globulins decreased in several of the hormone-treated patients in spite of the fact that they had an increase in antibody.

It is apparent that there can be no point of general agreement at the present time concerning the mediation of adrenal cortical hormones in either the synthesis or the release of antibody. However, it has been demonstrated that antibody is formed and released in the absence of the adrenal cortex. Under proper conditions of experiment, antibody synthesis can be markedly depressed by the administration of very large amounts of adrenal cortical hormones; cortisone and hydrocortisone have been shown

to diminish markedly the capacity of immunized animals t synthesize antibody This effect has not as yet been confirmed in humans, and it is believed that the dosages required to produce a suppression of antibody synthesis are far greater than those used either therapeutically or experimentally in human patients. It seems that a high level of circulating adrenal hormones must be attained pri r to the administration of the antigen in order to suppress antibody f rmation. If adrenal hormones are given even shortly after antigen is administered, relatively little suppression is brought about.

The author has analyzed this from the standpoint of dose-response phenomenon. Single graded doses of antigen were d ministered to inbred mice on a weight basis some of the immunized animals received daily graded doses of either ACTH or cortisone during the entire period f study Instead f the usual serial dilution method of establishing response, a technique was used whereby the extent of antibody suppression was graded according to a quantal response, that is, based upon a plus or minus appearance of antibody t a single dilution. The degree of suppression of antibody was determined for a very large number f animals at intervals of 4, 9 12, and 17 days f llowing administration f single dose of antigen.

These experiments revealed that the degree of antibody suppress on is directly d pendent n the relation of the amounts of antigen and hormone administered, i e., the greater the immunizing dose, the greater the amount of hormone required to suppress antibody Antibody is suppressed most effectively f r four days, and thereafter begins to appear despite continued hormone treatment. Complet suppression f antibody in the early period requires dosages greater than those which are, weight f weight, used therapeutically in human disease. In spite of lmost complete inhibition of antibody in the circulation, the incidence of anaphylactic shock is only moderately red ced, indicating that antibody is present in tissue when it cannot be demonstrated in blood. This evidence supports the suggestion of Mirick that "the beneficial effects of ACTH and cortisone in treating hypersensitivity and related diseases i man are not to be explained by suppressed antibody productions"

Animals producing antibody to ovalbumin were administered, at the point f maximum concentration, enough ovalbumin to reduce the titratable antibody to zero Subsequent cortisone ad ministration resulted in the rapid reappearance of antibody in the

circulati n, whereas the *noncortisone-treated* `ontr ls did not` manifest circulating antibody until 24 to 48 hours later The secondary response in the control animals, however p oduced higher titers than those f the cortisone-treated animals. This evidence seems t indicate that cortisone can bring about release of preformed antibody and can produce decrease in the rate of antibody synthesis, as shown by the diminished amount ppearing in the blood during the secondary response.

Effect at cellular level

Some investigators have suggested that reticuloendothelial cells are antibody producers others have supported the possibility that plasma cells and/ lymphocytes may be the main ite f synthesis f these proteins It is possible that all three f these cell types may play some role in the overall synthesis of antibody protein I any case, the mechanism by which adrenocortical hormones may suppress antibody synthesis is intimately related to the effect of these hormones on the cells which produce antibody

Reticuloendothelial cells, which incorporate antigen, undergo differentiation to arious types of cells such as the lymphocyte and the plasma cell. The ff pring, such as the stages of differentiation f lymphocytes and possibly plasma cells, actually carry out most f the synthesis f antibody protein. This process is comparabl to that f the synthesis f hemogl bin which begins in p oormoblasts and continues throughout the arious stages f differenti ation of erythroblastic cells. Thus, the direction o the type of antibody protein synthesized is predetermined in the reticula cell which phagocytized the antigen.

It is well known that there is tremendous inhibition of lymphocyt poiesis resulting from excessi adrenocortical hormone secreti n or administratio of the h rmones f th C-11 oxy or hydroxy-type. The essence f the problem would seem t be that th r types f agents capable f suppressing antibody formati n may act similarly F example, the well-known suppressi effect f x radiation on lymphocytopoiesis is accompanied by inhibition of antibody synthesis. Complete starvation induces an inhibition f lymphocytopoiesis which, oddly enough, is mediated by way f the adrenal cortex suppression of antibody synthesis lso accompanies thi type f suppression f lymphocyte f rmation. Other *agents that inhibit lymphocytopoiesis,* even in the absence f the adrenal cortex, as do the nitrogen mustards, also inhibit anti

body synthesis in the absence of the adrenal cortex. Pyridoxine deficiency even in the absence of the adrenal cortex, brings about the inhibition of lymphocytopoiesis and an accompanying inhibition of antibody synthesis. In each of these inhibitory responses it appears that if antigen is given before the suppression of lymphocytopoiesis there is less inhibition of antibody synthesis.

Several of these agents, e. g ACTH and x radiation, on the other hand, do not ignificantly diminish plasma cell formation. Many reports have verified the finding that ACTH actually en hances plasma cell formation. Plasma cells are not decreased by large doses of ACTH to the same extent as lymphocytes. For these reasons, in part, it would seem that much of the suppressive effect of the adrenal hormones n antibody synthesis is related to and is a consequence f the suppression of the formation of lymphocytes. It should be noted that there appears to be n question but that plasma cells as well as lymphocytes co tain antibody H weve the cellular content of this protein cannot be taken di rectly as an indication of the role of the particular cell in the synthesis of th protein. It should also be noted that several uthors have now cultivated lymphocytes in tissue culture and have demonstrated that these cells will synthesize antibody

There is some evidence that the adrenocortical steroids bring about release of preformed antibody The role of drenocortical steroid i bringing bout the catabolism f the antibody when it is delivered t the circulation is not well nd rstood. It is apparent, however that they do not greatly enhance the removal of this protein from the circulation. On the other hand, the evidence seems to be very clear-cut that the adrenal hormones, like other antilymphocytopoietic and environmental conditions, bring about a suppression of synthesis of antibody protein and inhibition of lymphocytic differentiation.

Discussion

DR. EHRICH This session on basic concepts of immunity and hypersensitivity is indicative of our sl w rate f progress in under standing and clarifying this extremely compl x and confusing subject. Terminol gy and definitions must be precise so that confusion is not compounded.

DR. CAMPBELL. Tw important areas of need for more under standing are (1) the nature of noncomplexing antibodies and their contribution to the early stages f immunization, and (2) the per

sistance of antigen. Many experiments directed at the latter problem have resulted in efforts to locate the original antigen. Since all antigenic materials are digestible, fragmentation must occu The task of localizing these fragments of necessity must be done within the body since degradation f antigen does not proceed in imila manner in in vitro experiments.

DR. DIXON As yet, we d not have adequate antigen tracer techniques to finally answer this question. On the one hand, anti genic fragments are often difficult to trace and on the other hand, we may detect foreign materials by our tracer techniques which re retained in tissues but are not stimulating antibody production. We are a long way from unraveling this problem.

DR. DOUGHERTY In discussing the persistence o retention f antigen I should like to cite the findings from an experiment with lymphosarcoma cells in mice. A lymphosarcoma tumo transplanted into a mouse previously immunized with tumor cells can be transferred to other mice for total of three passages.

DR. ROBERT A GOOD It is our view based on correlati morphologic immunologic studies that there is some truth in each of the current cellular theories of antibody production In the face of current evidence, some of it direct, ther can be no doubt that plasma cells produce or release antibody When cells f the body begin to produce antibody or gamma globulin, this physiologic function gains morphologic expression. Evidence obtained from morphologic studies indicates that in response to antigenic stimulation plasma cells can be derived from undiffe entiated reticulum cells, periadventitial mesenchymal cells, lymphocytes, monocytes and even microglia. Under rdinary experience with an initial injection of antigen plasma cells seem to derive largely f om the multipotent reticulum cells f the lymph node pleen and bone marrow Under more extreme stimulation as during the course f the secondary response particularly in the area f the local inflammatory process induced by exposure to pecific antigen to which an animal has previously been immunized, these cells may derive from lymphocytes, monocytes, microglia r periadventitial mesenchymal cells. As these various cells f mesenchymal origin begin to f rm antibody thei cytoplasm accumulates ribose nucleic acid, hence its basophilia. The nucleus becomes eccentric, probabl as a function of the secretory acti ity A clea area or hof develops in the perinuclear region as the mitochondria, Golgi pparatus and cytocentrum displace the ribose nucleoprotein of the cytoplasm, and the nucleus becomes that f a cell engaged in vigorou metabolic

activity These morphological features are the criteria by which the pathologist defines plasma cells It seems from our data then that many cell types, including perhaps even reticuloendothelial cells, may form antibody but that when they are actively engaged in this function they take n the morph logical appearance of plasma cells. With this view the plasma cell, derived from any source, becomes the morphological sign of antibody producti n.

Studies on the Mechanisms Involved in the Shwartzman and Arthus Reactions

The Shwartzman reaction

DR. GOOD Future research will doubtless bring understanding of the destructive processes associated with infection, allergy and hypersensitivity in precise physiologic and biochemical terms. Progress toward definition of tissue damage in this way requires controllable models of necrotizing processes which can be manipulated with facility and the manipulation of which promises to shed light on disease mechanisms in man. Such a model is the Shwartzman reaction. This is a two-stage reaction resulting in hemorrhagic necrosis of the skin. This lesion is produced whenever an intradermal injection of a gram-negative bacterial endotoxin is followed after a suitable time interval by an intravenous injection of the same or similar toxin.

A mild inflammatory response characterized by erythema, edema, and slight induration, which develops around the site of the initial intradermal injection of toxin, is called preparation. The sequence of events following the intravenous injection of endotoxin is called provocation. A minimum of eight hours is required for skin preparation, and the hemorrhagic necrosis occurs three to four hours after the intravenous injection of endotoxin. The Shwartzman reaction is immunologically nonspecific; preparation and provocation can be carried out with filtrates of unrelated microorganisms.

Characteristics of prepared skin

Morphologically the prepared skin at the site of intradermal injection of endotoxin has the appearance of inflamed skin. Infiltration with polymorphonuclear leucocytes is noted with conspicuous perivascular cuffing. Some mononuclear cells may be observed. Blood vessels for the most part are patent. In some capillaries and venules, margination and diapedesis of leucocytes may be seen. There are no morphological findings that differentiate preparation for the Shwartzman reaction from an ordinary in-

flammatory response. However prepared skin does not stain in response to an intravenous injection of trypan blue, in contrast to normal skin which turns blue, or to an area of inflammation which shows enhanced permeability to vital dyes.

Metabolic abnormalities can be demonstrated in prepared skin Intense aerobic glycolysis with consequent accumulation of lactic acid and local lowering f pH have been described. Substances such as f reign proteins giving rise to an inflammatory reaction, but incapable of preparing the skin, do not enhance lactic acid production t the same degree as do gram-negative bacterial endotoxins.

Inhibition of the reaction

Inhibition of the Shwartzman reaction in rabbits by pretreatment with nitrogen mustard, x ray r benzene (agents known to produce leucopenia) demonstrates the essential rol f poly

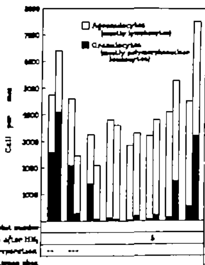


Figure 8. Relation of inhibition f Shwartzman reaction to fall in granulocytes ad ministration f nitrogen mustard. Reprinted from the Journal of Experimental Medicine 93:49 (1951)

morphonuclear leucocytes in this process. Nitrogen mustard in a dose of 1.5 mg. per kg. of body weight produces a granul penia maximal in 72 t 96 hours. The Shwartzman reaction is regularly inhibited during the period of maximal leucopenia but may occur both prio to the fall in granulocytes and following their reappear ance in the circulation (figure 8)

That inhibition of the Shwartzman phenomen n is not due to some other toxic effect of nitrogen mustard can be demonstrated by

protection of the femoral marrow from its action. A clamp placed on the abdominal aorta of the rabbit for a fi e minut period dur ing and after the injection of the mustard all ws f r fixati n f the nitrogen mustard by those areas of the body above the clamp, sparing the femoral marrow below While these animals show evidence of toxicity to nitrogen mustard they do not dev lop granulopenia. The prepared skin sites give characteristic Shwartz man reaction (tabl 2)

Blocking the granulopenia induced by benzene by simultaneous treatment with sulfapyridine allows these rabbits to exhibit a Shwartzman reaction. Presumably because of the neutr penia produced, the Shwartzman reaction does not occur in animals treated with benzene alone.

The increased glycolysis and lactic acid production of skin prepared for the Shwartzman reaction are diminished in rabbits made leucopenic by pretreatment with nitrogen mustard. Prepared skin, impermeable to trypan blue in the classical Shwartzman reacti n. stains with this v tal dye in animals having polym rphonuclea leucopenia. Thus the polym rphonuclear leucocyte seems t be essential for most f the changes described as characteristi of prepared skin.

Leucocyte changes

P lymorphonuclear leucocytes may lso play a vital role in the devel pment of the hemorrhagi ecrosis that f llow the p ovoking injectio f toxin. Immediately after an intravenous injection f toxin, thrombocytopenia nd granulopenia are bserved. This phenomenon is attributed t the devel pment f adhesiveness f leucocytes and platelets, with remo al of these f rmed elements from the circulati as clumps o pl gs in the capillary bed f the lungs and other rgans. As Stetson has shown, the capillaries and venules in the prepared skin site become occluded by leucocyte-platelet thrombi, and the necrosis f the essel wall as subsequent hem rrhagic necrosis seems to be function f this event.

Effect of anticoagulants

The concept that thrombus formation is essential as stage in the developement f the Shwartzman reaction is supported by study f the ffect f heparin on the reaction. Intravenously administered aqueous heparin, or subcutaneously injected depot heparin in quantities t produce incoagulability f rabbit blood for four hours,

Table 2

Susceptibility to the Shwartzman phenomenon, following treatment with HN_2, of rabbits in which the femoral marrow had been protected against the HN

	Rabbit No.	Total leucocytes		Polymorphonuclear leucocytes		Intensity of Shwartzman phenomenon
		Before skin preparation	Before intravenous challenge	Before skin preparation	Before intravenous challenge	
		cells/cmm.	cells/cmm.	cells/cmm.	cells/cmm	
Protected" rabbits	1	6600	8550	3300	2140	++++
	2	6850	4550	5150	2080	++++
	3	5500	3550	4350	2130	++++
	4	4900	6400	2690	3200	++++
Sham-operated rabbits	5	4250	3650	340	73	—
	6	2110	2550	21	51	—
	7	3600	3750	410	211	—

++++ indicates development of typical hemorrhagic necrotic skin lesion following the intravenous challenge — indicates absence of any hemorrhagic-necrotic reaction following the intravenous challenge

completely blocks the development of the Shwartzman reaction when given at the time f provocation (table 3)

Administration of heparin during the period f preparation is without effect. In the doses used it did not affect the leucocyte r platelet count nor prevent the polymorphonuclear leucopenia that f llows intraven us injection of toxin. While heparin had profound influence on the Shwartzman reaction it did not inhibit the lethal effect of a single large dose of intravenously injected toxin.

Table 3

P evention of local Shwartzman eaction with hepari

Group	Rabbits injected	Positive reaction
No heparin	30	29
Heparin I.V	18	2

Other anticoagulants such as Tromexan® and dicumarol are lso effective in blocking the Shwartzman reaction. These observa tions upport the belief that vascula occlusion constitutes part f the mechanism involved in the development f hemorrhagic necrosis f this type.

Vascular spasm

Vascular spasm may also be facto in the development f the Shwartzman reaction. The injection of adrenalin, 1 ml f 1 10,000 dilution, into the skin and subcutaneous tissue surrounding a prepared skin site results in lesion virtually identical to the Shwartz man reaction. Hemorrhagic necrosis does not occu if the injection of adrenalin is made intra enously subcutaneously in n are far removed f om the site f preparation.

The Arthus reaction

The Arthus reaction, like the Sh rtzman reaction, is skin lesion characterized by hemorrhagic necrosis. Although produced by entirely different methods the bservations of Stetson indicate that this phenomenon also invol es the following mechanisms

1 Peri ascula accumul tion f polymorphonuclear leucocytes in the i jected skin area.

2. Aerobic glycolysis at the site of antigen injection.
3. Adhesiveness of leucocytes and platelets from contact with the provoking agent.
4. Leucopenia following the injection of the provoking agent r antigen.
5. Leucocyte-platelet thromb in the capillaries and venules of the injected area.
6. Hemorrhage from vessels occluded by cellular thrombi.

Rabbits previously sensitized to ovalbumin and prepared for the Shwartzman reaction by the intradermal injecti n of meningococ cal t xin sh w hemorrhagic necrosis 24 hours after an intradermal injection of ovalbumin, t the site of the initial ovalbumin injecti and t the site of injecti n f meningococcal toxin.

Effects of cortisone and ACTH

Contrary to other fi dings reported, cortisone given in any dose range t rabbits from o stock col ny does n t inhibit the Shwartzman phenomenon. Whil ACTH does result in slight alter

Table 4

Effect f cortisone and ACTH on the Shwartzman eaction

Group	Dose mg./kg. body weight	No. rabbits	No. positive
Cortisone (daily for 4 days)	25	10	9
	20	8	8
	10	5	4
	8	6	6
	5	6	5
	2	6	6
	0	16	15
ACTH			
(every 6 hours f 4 days)	10	8	8
(every 9 hours for 4 days)	8	6	6
	5	5	5
	2	6	6
(every 8 hours for 3 days)	40	6	5
	0	22	1

ation in the gross features of the reaction it does not prevent the local or generalized Shw rtzman reacti n (table 4) Instead, in cortisone-treated rabbits a primary hemorrhagic necrosis occurs following an intradermal injection of toxin without a second intravenous injection f endotoxin.

The primary hemorrhagic reaction to toxin in cortisone-treated rabbits is prevented by p etreatment f rabbits with nitrogen mustard by treatment with heparin. In its morphologic features this skin lesion seems to evolve thr ugh the same mechanisms that are involved in both phases f the Shwartzman reaction. Following this observation it was observed that single intra enous injection f t xin in cortisone-treated rabbits results in the dev lopment of hemorrhagic necrosis in the kidneys. Normal rabbits rarely manifest this lesion after a single injection f the toxic material. Bilateral cortical necrosis f the kidney is characteristic f the generalized Shwartzman reaction, and was the lesion regularly produced by a single injection of toxin in cortisone-treated rabbits. This cond tion was not so regularly observed in ACTH-treated rabbits f llowing a ingle injection of toxin. Whereas cortisone interferes with the shock producing and lethal action f gr m-neg tive bacterial endot xins, both cortisone and ACTH enhance susceptibility or rabbit t the necrotizi g action of t xin

Effect of thorotrast

Some rabbits ppear to be more resistant to the damaging effects f endotoxins than others, and the repeated injection f small doses results in the development f a high degree f resistance to the Shwartzman phenomenon. Reticuloendothelial blockade produced by the intravenous injection f thorotrast abolishes both the naturally occuring state f resistance and acquired resistance

Susceptibility f n rmal abbits to the lethal effects f toxin can be increased more than 1000-fold by prior injection f thorotrast. These observations are interpreted as evidence that the reticuloendothelial system plays an important role in the body's defense gainst toxins of this las

Implication of the Generalized Shwartzman Reaction

Dr. Lewis Thomas The generalized Shwartzman reaction occurs in the rabbit when tw intravenous injections of gram-

negative bacterial endotoxin are spaced 18 to 24 hours apart. As in the local Shwartzman reaction, it is essential that there be two injections, and that adequate time be allowed between the injections. The reaction is not due to the cumulative effect of the two injections, since it fails to occur with a single dose of much larger quantities of toxin than is effective in two divided dose. A characterizing manifestation is bilateral cortical necrosis of the kidneys, a lesion that appears to be initiated, within four hours after the second injection of toxin, by occlusion of the glomerular capillaries with masses of homogeneous eosinophilic material resembling fibrinoid.

The presence of circulating polymorphonuclear leucocytes appears to be necessary for the reaction. In rabbits with leucopenia caused by nitrogen mustard, bilateral cortical necrosis of the kidneys cannot be produced. When leucopenia is prevented by shielding the femoral marrow from the action of nitrogen mustard, renal necrosis occurs as in untreated rabbits. The role of polymorphonuclear leucocytes in the reaction cannot be explained.

Heparin prevents the occurrence of the generalized Shwartzman reaction, apparently by preventing the deposition of occluding material within the glomerular capillary lumen.

Cortisone does not protect animals against the reaction. On the contrary cortisone treatment causes the animals to react to a *single* injection of endotoxin by developing renal cortical necrosis. This observation suggests that cortisone may interfere with a normal protective mechanism for the detoxification of such substances. In view of the known morphologic effects of cortisone on tissues of the reticuloendothelial system, the possibility that the altered response to toxin might be mediated by an effect on this system was investigated.

An intravenous injection of colloidal materials known to be taken up by reticuloendothelial cells, including thorotrast, trypan blue, colloidal iron saccharate, and colloidal carbon, was followed by a single intravenous injection of endotoxin. The outcome was similar to that in cortisone-treated rabbits, one dose of toxin resulting in bilateral cortical necrosis of the kidneys in a high proportion of the animals. Reversal of the order of injection, with the colloidal substances given a few hours after toxin instead of before, produced no kidney lesions.

Limitation of protective function of reticuloendothelial system

On the basis of these and other observations the following

hypothesis was constructed to account for the necessity for two intravenous injections f endotoxin in the generalized Shwartzman reaction. The animal responds to the first dose of toxin with a protective mechanism that involves the functioning of cells of the reticuloendothelial system. As the result f saturation of capacity this system becomes temporarily unabl to repeat its function, and the second dose f endotoxin is free to act d rectly on other tissues such as the kidney which are not affected in the normal animal A similar state of vulnerability is caused by cortisone, thorotrast, trypan blue, and colloidal iron and carbon in animals treated with these materials the protective function of the reticuloend thelial system is temporarily impaired, and a single dose of toxin causes renal necrosis.

The occurrence f a similar ituation in animals with systemic infection by group A hemolytic streptococci has been investigated When streptococcal infection is followed by a single injection f endotoxin severe generalized Shwartzman reactions ar produced, accompanied by fibrinoid necrosis f the coronary arteries, which resembles the rterial lesions commonly ascribed t "hypersensitivity

A Pattern of Tissue Reaction in Hypersensitivity

DR. CAROLYN F PIEL Increased knowledge f the role f hypersensitivity in human disease has greatly stimulated endeav to produce experimental models of such diseases. These models would then not only be available f investigation f therapeutic gents, but permit detailed study f the pathogenesis of clinically lusi entities. Outstanding in this respect are the innumerable observations involving ttempts to produce renal disease in laboratory animals.

Of the variety of methods employed in the production f experimental nephritis, bacterial injections alone or bacteria mixed th homologous kidney emulsions has been widely utilized. The results has been singul rly unrewarding and difficult t reproduce

Heterologous serum in experimental renal disease

Another method that has proven m re profitable inv l es the use of heterologous proteins. Intravenous injection of whole horse

or bovine serum or the purified fractions, globulin and albumin, produces a renal disease that many observers have compared with human glomerulonephritis. The experimental disease is characterized by a latency of 7 to 10 days between the time of injectio and the onset of symptoms including proteinuria and pathologic findings such as proliferative changes of the glomerular tuft. I addition, animals injected with heterologous protein have lesions elsewhere in the body periarteritis, myocarditis and valvulitis.

By applying special histochemical techniques t kidneys removed from rabbits injected with whole horse serum, the proliferati change is noted to involve the mesenchymal tissue of the glomerulus, which lies between the capillary loops, rather than endothelial cells. This tissue reaction is not typical of human glomerulonephritis but rather f a generalized mesenchymal reaction such as is seen in serum sickness.

The renal lesions produced by heterologous protein depends on the ability of the antigen t reach the kidney the ability of the recip ent animal to produce pecific antibody and the occurrence f antigen-antibody reaction in the glomeruli. Purified album n produces less renal disease than globulin, probably because its smaller molecular size allow considerable removal from the bloodstream prior to reaching the kidney Hyaluronidase likewise depresses the renal lesions by facilitating the removal of antigen from the blood stream. Cortisone, x radiation, nitrogen mustard, colchicine and low protein diet all inhibit the occurrence of nephritis, probably on the basis f their antiphlogistic effect and their interference in production of antibody

Use of nephrotoxic serum

Nephrotoxic serum prepared by repeated injections f em lsi fied kidney into animals f another pecies produces ren l disease on injection into animals f the donor species. Nephrotoxic serum is highly species- and organ-specific. The antigenic portion of the kidney resides in the membranous section of the glomerulus. Adsorption of such sera by kidney emulsion *in vitro* produces a loss in nephrotoxicity Localization of the site of a tigenicity was made possible through observations demonstrating that cortical emulsions produce renal disease more readily than medullary emulsions. By differential centrif gation the gl merular fraction was found to be 20 times more antigenic than a tubular fraction. F rther subdivision of the glomerular fraction localized the antigenic sit to the membranous portion. Kidney emulsion incubated with trypsin results

in a clear stable soluti n, nonantigenic but capabl f inactivating nephrotoxic serum. It is postulated that th antigen is protein-bound polysaccharide or lipid.

Further confirmation f the site f antigen localizati n has been obtained from tagged antibody studies Mice njected w th radio-iodinated antikidney globuli show localization f radioacti ity in the gl meruli. Fluorescein-tagged globulin from serum prepared using whole kidney produces fluorescence f the ytoplasm f the epithelium of the glomerular capillaries and cortical tubules. Globulin prepared from serum foll wing gl merular tissue jection and tagged with fluorescein results in fluorescence f gl merular struc tures only

Nephrotoxic fowl serum

Nephrotoxic serum prepared in f wl duck p oduces in dogs, rabbits, guinea pigs and rats a disease with two distincti features, (1) a latency period f one week before onset of ymptoms, and (2) hematuria. Rabbits injected with nephrotoxic duck serum may fix the duck serum in their kidneys. Fi t seven da later the forma tion of rabbit and duck serum antibodies, may interact with th duck serum fixed in the abbit kidney and result in nephritis. Oliguria, proteinuria, cylindruria and moderate edema characterize the clinical course of this disease. Some animals devel p hyper tension, urea retention, and decrease in glomerular filtration rate

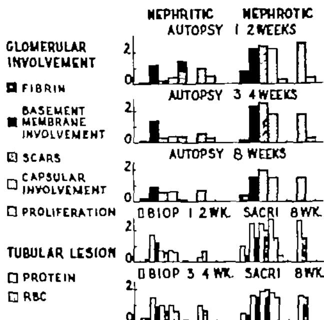
Nephrotoxic rabbit serum

Renal disease produced in rats f llowing the injection of nephrotoxic rabbit serum differs from that produced by nephrotoxic duck serum in immediate onset f disease and lack f hematuria. By varying the potency f the rabbit serum tw types of disease response can be btained. One has proliferati hanges comparable to human glomerulonephritis, occurring after latency f one week; the other occurring immediately fter serum injection, has basement membrane involvement comparable t nephrosis.

The results of study designed to establish whether r not these two types f tissue response remain distinct entities merge int one another are summarized in figure 9 The changes studied are graded one to four plus, and the influence f biopsy procedures on the rate f progression are indicated In general, the histologic changes are less se re in the nephritic group. In the early week f study the nephritic group shows mo proliferation nd red blood

cells in the tubular lumena. Basement membrane involvement in the nephrotic group tends to subside by the eighth week. Biopsy at one to two weeks intensifies the basement membrane changes. Biopsy at three to four weeks has less influence on outcome.

The influence of diet, stress, enzymes, and cytoxic agents on the course of this disease in rats has been difficult t evaluate. Recovery



Figur 9 Average histologic and urinary changes in the kidneys of rats given two levels f nephrotoxic rabbit serum.

f rats given low pr tein diet has been recorded. Nitrogen mustard has been reported to depress the proteinuria f nephrotoxic serum disease. Cortisone and ACTH dministration pri to or following the injection f nephrotoxic serum depresses edema, protei uria, degenerative and proliferative changes. Cortisone in doses greater than 2.5 mg. per 100 gm. of body weight aggra tes the disease

Discussion

Dr. Good Is the formation of fibrinoid material in the glomerul f animals given nephrotoxic serum i hibited by heparin d ministration?

DR. PIEL Studies f nephrotoxic disease by Dr Ehrich indicate that heparin will prevent the production f thrombi.

DR. GOOD Does such inhibition alter the severity of the renal lesion? Some of the thickening of the basement membrane may arise on this basis.

DR. PIEL: Inhibition f fibrinoid formation sh uld alter the severity of the renal lesion. Animals that have been biopsied reflect a more severe disease pr bably due to the nonspecific production f fibrin thrombi.

DR. GOOD We vacillate between calling this material fibrin and fibrinoid. Rarely on histochemical study does it have a fiber structure. Perhaps it is in a stage of fibrin development.

DR. EHRICH We don't know the difference between fibrin and fibrinoid. Relative t the use f heparin we have noted that heparin eliminates gross edema completely

DR. GERMUTH Does not the very fact that heparin inhibits the deposition of material suggest that it is fibrin? When blood capillaries undergoes thrombosis, fibrin is ften noted.

DR. EHRICH I experimental ephrosis as seen in the rat we do not necessarily have fibrin. The material accumulating on the basement membrane seems to be serum protein.

Influence of Cortisone on the Pathogenesis of Anaphylactic Hypersensitivity

DR. FREDERICK G GERMUTH, JR. Allergic phenomena like the immune processes f acquired resistance are mediated through ntibodies. Allergy ppears when the interaction f antigen and ntibody produces injury to cells f the animal. Immunity occurs when the interaction alters microbial cells. Allergy and immunity are theref re different bi logic expressions f the same immunologic phenomenon.

Allergy and fixed antibody

Allergy may manifest itself in ariety of w ys. I some forms f llergy such as acquired hemolytic anemia nd idiopathic thrombocytopenic purpura, antibodies re directed against the surfaces

of specific tissue cells which have been modified by the offending antigen. In drug allergy of the contact dermatitis type and in the allergy that follows infection, it would appear that antibody is carried in or on lymphocytes. Antibodies in allergy may be more or less firmly attached to cells, and free antibody in the serum may be slight or absent. In all of these instances, the result is damage to a specific cell of the body

Allergy and circulating antibody

Exposure of the animal body t foreign protein antigen evokes the f rmation of circulating plasma antibodies which are not directed against any particular cellular component of the body This type of allergic state induced by the formation of circulating antibodies is known as anaphylaxis. Here the interaction of circulating antibodies with antigen in the tissues leads to contraction of smooth muscle and damage to the cardiovascular system. These alterations are familiar in the form of asthma and allergic arteritis in man, and i the form of anaphylactic shock, the Arthus reaction and serum sickness in experimental animals.

Anaphylactic shock results from the intravenous injection of antigen into a sens tized animal extremely small amounts of antibody are required to produce shock.

Intracutaneous injection of antigen into a sensitized animal is usually followed by local inflammatory reaction characterized by redness and edema and, in its more severe f rm, hemorrhage and *necrosis. This symptom complex is known as the Arthus reaction.*

Large quantities of antigen such as foreign serum injected i travenously into a normal animal may be followed, after an incubation period of several days, by the nset of fever and the appearance of severe damage to the cardiovascular and renal tissues. This state of protracted anaphylaxis is known as serum sickness.

Effects of hormones

The administration f daily doses of cortisone, ACTH or *cholesterol (5 mg. per kg of body weight) to rabbits injected* daily intracutaneously with 1 mg. of crystalline egg albumin produced marked suppression in the Arthus reaction in those rabbits receiving cortisone. Serum antibody concentrations were reduced by the administration of ACTH while cortison produced almost complete i hibition of antibody response. Failure of ACTH t alter the Arthus reacti n seems dependent pon its inabil ty to suppress anti

body below a level of 40 micrograms of antibody nitrogen per ml. of serum.

Since cortisone exhibits anti-inflammatory acti n, its inhibiting effect on the allergic state may be related to an altered skin reactivity Rabbits treated with cortisone and tested for passive Arthus reaction from antibody administered directly int the skin or intravenously show no effect of cortisone on the Arthus reaction (figure 10) These findings led to the conclusion that the inhibition of the active Arthus reaction by cortisone is effected by hormonal suppression of cir culating antibody Since circulatory antibody concentration can be reduced by either decrease in antibody synthesis r an increase in

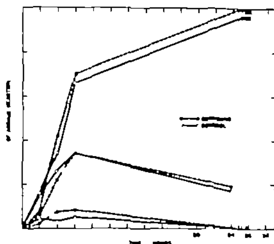


Figure 10. Effect f cortisone on passive Arthus reaction. Rabbits given antibovine albumin rabbit serum intravenously and skin-tested with (1) 0.01, (2) 0.1, and (3) 1.0 mg. of bovine albumin at one-half hour intervals.

the rate f antibody destruction, the mechanism by which cortisone administration alters antibody level requires further study As shown in figure 11, cortisone is without effect on the rat f disappearance f antibody from the serum f passively sensitized rabbits, *suggesting that cortisone suppresses antibody formation.*

The mechanism by which cortisone suppresses antibody f rmation is bscure. Decreased antibody formation may ha some relation to the extensive atrophy f the lymph id tissue found in ACTH and cortisone-treated animals.

Large doses of cortisone are without effect on either active or passive anaphylactic shock in the guinea pig Since the quantity of antibody required for shock is small, inhibition of antibody synthesis by cortisone does not inhibit shock.

Serum sickness

Advances in the understanding of the relationship between

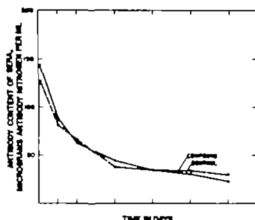


Figure 11 The influence f cortisone on the rate f antibody disappearance from the serum f passively immunized rabbits.

Table 5

Experim ntal lesions nduced in ebbits 12 days after intra enous bovine albumin

Lesion	Percent
Acute glomerulitis	80
Focal accumulation of leucocytes in intima f pulmonary arteries or scending aorta	72
Endocarditis (aortic and mitral valves)	54
Necrotizing arteritis (predominantly coronary)	28

tissue lesions and immunologic cha ges associated with serum sickness are possible when use is made of chemically homogeneous antigens instead of mixt res. Rabbits given single intravenous in jection f crystalline bovine albumi (0.25 gm. per kg f body

weight) show a high incidence of cardiovascular and renal lesions (table 5) Granulomatous lesions re observed in the spleen and lymph nodes of more than one half of the e perimental animals.

The relationship among the time of onset f tissue lesions, the clearance of antigen from the serum, and the development f free antibody is shown in figure 12. Bovine albumin is eliminated in three phases and is followed by the appearance f antibody Tissue

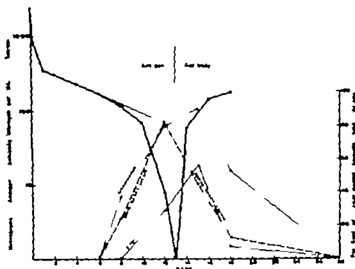


Figure 12. Relationship between the disappearance of antigen and appearance f antibody to the onset of tissue lesions in experimental serum sickness.

lesions are first observed during the third or immune phase of antigen limination, when antibody formation is proceeding in the tissues. About the time that the immune phase is complete, tissue lesions are most severe and f highest incidence. With the ppear ance of free serum antibody healing occurs. These temporal rela tionships indicate that the tissue lesions result from antigen-antibody combination occurring when antigen reacts with antibody formed fixed to tissue.

The Arthus reaction, which results from the ombination f circulating antibody with antigen introduced into the skin, occurs after the ppearance of free antibody at a time when tissue lesions are regressing

Cortisone and serum sickness

The action of cortisone on experimental serum sickness is summarized in table 6. Rabbits given a single intravenous injection of 0.5 gm. of bovine albumin were sacrificed on the 12th day. One

Table 6

The effect of cortisone on the induced hypersensitivity of serum sickness in rabbits

Effect	Number showing effect	
	Control	Cortisone-Treated
Total number of animals	25	23
Antibody	7	5
Arthus reaction	5	3
Subendothelial infiltrations	18	0
Necrotizing arteritis	7	0
Endocarditis	13	1
Glomerulonephritis	20	4
Lesions		
Spleen	20	16
Lymph node	8/16	0/6

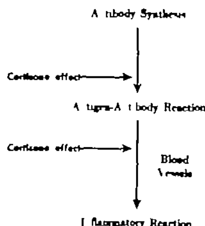


Figure 13. Possible roles of cortisone action in the allergic state.

half the group received a daily dose of cortisone, 5 mg. per kg. of body weight during this period. Cortisone did not inhibit antibody formation or the Arthus reaction under these circumstances, although the incidence and severity of visceral lesions were markedly

modified. Granulomatous lesions in the spleen of cortisone-treated rabbits were smaller and fewer in number. The mechanism by which cortisone inhibits visceral allergic lesions without suppression of antibody production is not clear from this experiment.

Cortisone may inhibit anaphylactic allergy by two distinct means, (1) suppression of antibody production, and (2) suppression of the damaging effects of antigen-antibody union. Many clinical cases achieving therapeutic benefits from cortisone seem to involve the latter mechanism while suppression of antibody production is not usually observed (figure 18).

The Role of Complement in Hypersensitivity

DR. FREDERIC C. MOLL. Since the fixation of complement is used daily in diagnostic laboratories as an index of antigen-antibody union, the functions of complement *in vivo* should be better understood. However its instability, complex structure, and varying behavior under similar conditions make description of its function difficult. The most consistent *in vivo* finding is the diminution in the level of circulating blood complement accompanying some pathological states ascribed to antigen-antibody interaction. Low complement titers in acute nephritis, rising to normal during convalescence, and low complement titers during serum sickness have been described. In the absence of hepatic disease the finding of low serum complement *in vivo* is regarded as an index of antigen-antibody reaction.

To delineate the relationship of complement to antibody formation, a group of rabbits were given a single large injection of heterologous serum, and serial antigen-antibody and complement studies were performed. Uninjected control animals show no change in serum complement level, whereas rabbits receiving bovine gamma globulin manifest a pattern typical of many antigens and of many other species. Eight to 10 days after injection the blood complement level falls, rising in two to four days, at which time circulating antibodies can be demonstrated. Animals treated with x-ray, nitrogen mustard or cortisone in doses large enough to suppress antibody formation do not manifest a decrease in serum complement concentration.

Since pathological lesions attributable to hypersensitivity and

complement depression have been described only in animals given large doses of antigen, the relationship of quantity f antigen to antibody response requires consideration.

By the use of graded amounts of antigen it can be demonstrated that those animals receiving the smallest amount of antigen show less variation in serum complement and have fewer tissue lesions. Circulating antibody titers, however are as high in those animals given the smaller amounts of antigen as in those given the usual large amounts. While there is apparently no correlation between the symptoms of serum sickness and the appearance of circulating antibodies in clinical medicine, experimental animals behave differ ently The les ons of hypersensitivity can be demonstrated only when antibody is present.

Administration of ACTH prevents neither antibody formation no the characteristic f ll in serum complement, but does prevent the occurrence of lesions. Since complement level decreases, antigen-antibody interaction must occur with ACTH in some manner which protects the cell from damage. Cortisone administered to the rabbit prevents lesions by interference with antibod formation, as evi denced by failure to find precip tin in the irculating blood, and failure to observe any alteration in serum complement level.

During an acute episode of serum sickness change in serum complement may be detected in many cases. The host reaction in general cannot be predicted except that it appears to be related to the amount of antigen dministered. Anaphylactic states in man, typical in asthma, may occur without demonstrable change in serum complement titers.

Discussion

DR. THOMAS I there an effect of cortisone on the tissue distribution f antigen? Is there less more antigen in the heart or kid ey during cortisone treatment? What happens to animals given cortisone or ACTH for a short period, with subsequent antibody formation? The amount of foreign p otein deposited in the tissues may be more critical than y change occurring in the circulatio

DR. DIXON Serum protein antigens existing *in vivo* equilibrate with ll cells to which they have access. The concentration within tissues parallel that within circulating blood plasma, so that the blood level reflects tissue levels. We ha never found selective re tention or concentration of these antigens in any tissue.

DR. DOUGHERTY The administration of horse serum t mice.

followed by the administration of ACTH or cortisone to suppress antibody formation foll wed by a period of no hormone administration, results in the rapid development f lesions in the heart and lungs, which differ from the usual serum lesions. Sloughing of bronchial epithelium without inflammatory signs is noted. A non-inflammat ry myocarditis with degenerative changes f the myocardium is evident. Three r four day later an inflammat ry phase sets in.

Collagen Diseases

Dr. Ehrich The term collagen diseases designates a group of maladies characterized morphologically by systemic involvement of connective tissue. The concept of a systemic disease presupposes the presence of a b logical system, and connective tissue is such a system. Made up f an association of fibroblasts and collagen fibres held together by an amorphous ground substance, connective tissue functions as supporting tissue. Interposed between the parenchymes and the blood and lymph vessels, it also serves as a site f transfer of metabolites and plays an important role in salt and water balance.

The recognized collagen diseases are rheumatic fever rheu matoid arthritis, lupus erythematous disseminatus, generalized scleroderma dermatomyositis, serum sickness and polyarteritis. Glomerulonephritis is not included among these diseases because it is not a systemic disease of connective tissue. Since the heart and blood essels, and sometimes other elements of the mesenchyme, are affected, it is better to define the collagen diseases morphologically as systemic diseases of the vascular-connective tissue.

If it is true, as postulated in this paper that these diseases are dysgammagl bulinemias pathogenetically the involvement of the vascular system is a condition *sine qua n n* because the noxious agent, gamma globulin, has t pass through this system bef re it can contact the connective tissue

Distinguishing characteristics

The collagen diseases re characterized linically by their intermittent course and tendency t hronicity Chemically they are distinguished by a depression f the serum albumin and f the collagenase inhibito during acute stages, and by rise in fibrinogen, alpha globulin, gamma gl bulin, glycoprotein, the C-reactive protein and the non-specific hyaluronidase inhibitor during acute

and subacute stages. Morphologically mucoid degeneration, fibrinoid degeneration and necrosis, gran l ma formation and fibrosis (sclerosis) are common findings.

The chemical alterations observed in collagen disease appea t be unspecific manifestations indicati e of liver and vascular connecti tissue injury The morphological changes represent in tial injury reaction to injury and repair Th rise in gamma globulin appears t play a key role in the pathogenesis f lesions characteristic of collagen disease. A pecific reaction, the elevation in gamma globulin is not the result f tissue damage, but is rather due to plasma cell proliferation in the diseased connective tissue and bone marrow

Etiology

A common etiologic rigin for the collagen diseases cannot be found in thei ltimate causation. Such states as serum sickness, polyarteritis, and rheumatic fever are wholly or partially allergic in nature with n evidence f an llergic state found for others. There is no sound basis fo a belief that all collagen diseases are due t allergy The concept that collagen diseases represent failure f adaptation with an increase in pituitary and adrenal cortical function, has not been confirmed.

The common denominator is apparently the hypergammaglobulinemia due t plasma cell proliferation. I serum sickness, polyarteritis, and rheumatic fever antibody becomes injurious when combined with specific antigen. In l pus pathological gamm globulin, the L. E. factor is active in the absence f ex ngenous antigen. This situation seems to exist in rheumatoid arthritis and may be found eventually in generalized scleroderma and dermatomyositis.

The cause of collagen disease appears to be the production f abnormal gamma globulin from plasma cells. These proteins ci culate through the vascular bed, pas ing into vessel wall to become deposited there, and through them to the supporting connectiv tissue where localized ites f tissue inj ry can occur

Autoantibodies in Hematologic Disorders

DR. ROBERT EVANS: Autoantibodies constitute the serologic bn rmality that appears responsible f most cases f acquired

hemolytic anemia, of idiopathic thrombocytopenic purpura and some instances of unexplained neutropenia. Whether antigens in the affected cells are involved in the formation of antibody lik agents is not established. Injection of red cells into rabbits with or without adjuvants o foreign protein has been foll wed by evidence of autosensitization in some of the animals. Positive antiglobulin serum tests a e obtained transiently in rabbits injected intraperitoneally with the animal's wn blood.

Since red blood cells of any donor are hemolyzed when transfused into a patient with autoimmune hemolytic disease, the autoantibodies of hemolytic anemi are considered t lack specificity Recently the autoant body of one patient with autoimmune hemolytic disease was shown to have a specificity for the e antigen. While this specificity does not prove that the e factor in the patient s cells was responsible for evoking the antibody initially it does provide supporting evidence.

Drug and virus induced disease

In thrombocytopenic purpura caused by Sedormid® a mechanism of antibody production with important implications fo autoantibody formation can be demonstrated. The sera of patients with Sedormid sensitivity lyse platelets *in vitro* in the presence of Sed rmid. It is postulated that following ingestion of Sedormid a Sed rmid platelet conjugate is formed which is antigenic. In some ndividuals this conjugate evokes antibody that lyses platelets only when they are combined with Sedormid. The hypothesis that foreign substances such as virus particles or medicament may form conjugates with cell proteins that provoke antibodies which then sensitize the cell is attracti e, but unproven at present.

The observati n that certain cases of acute hemolytic anemia are due to virus sensitization of the red blood cell, forming the bridge between cell surface and antiviral globulin, is as yet unconfirmed. In a patient with viral pneumoni and autoimmune hemolytic disease studied recently we failed to demonstrate the presence of virus by lectron microscope photographs nd egg embryo transfer technique.

Properties of autoantibodies

Although all autoantibodies active in a t immune hemolytic disease are observed t sensitize red blood cell t ormal body temperature and pH, they are found to v ry in many other properties.

The variation in behavior of red blood cell autoantibodies is indicated by the many methods required for their demonstration. A few produce complement hemolysis, or agglutinate sensitized cells in saline at 37 C. Others agglutinate sensitized cells in a colloidal medium, but for most of them the antihuman globulin serum technique is required to demonstrate their presence on the patient' cells.

In a study of 34 patients with autoimmune hemolytic disease (table 7) only one had a demonstrable hemolysin in the serum for his own group O cells and the other group O cells tested. Although heat inactivation of the patient' serum prevented hemolysis of normal red blood cells incubated in the serum, these same cells

Table 7

Variations in demonstrability of autoantibodies in 34 patients with autoimmune hemolytic disease

Hemolysin in serum disappearing with heat inactivation	1
Agglutinin in serum active at 37 C in saline	1
Agglutinin on patients' cells reactive in colloidal media (30% beef albumin)	12
Agglutinin on patients' cells reactive in antiglobulin serum	
Strongly positive	30
Weakly positive	2
Negative	

showed a positive antiglobulin serum test, indicating that sensitization by antibody had occurred in the absence of complement. The hemolytic property of this patient's serum indicated a qualitative difference in the nature of the autoantibody.

The red blood cells of two patients failed to agglutinate in antiglobulin serum. The red blood cells of one of these showing a negative antiglobulin serum test, were agglutinated when centrifuged in 30 percent beef albumin, indicating the presence of antibody. An antiglobulin serum prepared by injecting the patient serum into rabbits produced a positive antiglobulin serum test with the patient' cells. It was concluded from these observations that the antiglobulin sera prepared by injecting normal human serum into rabbits did not contain antibody for the particular protein in the patient's serum responsible for sensitization of the patient's red blood cells.

In the second patient serological abnormalities were not demonstrable on the cells or in the serum by any technique. Normal

red blood cells transfused into this patient were destroyed at a rate equal to the rate of destruction of the patient's own cells, although isoantibodies as well as autoantibodies could be detected neither before nor long after the transfusion. Removal of a 250 gm. spleen that showed no evidence of phagocytosis or hypersequestration resulted in complete remission of the disease. The possibility exists that red blood cell destruction in this patient was caused by an autoimmune process even though a serologic abnormality was not demonstrable. An antiglobulin serum produced by injecting this patient's serum into rabbits did not result in a positive test.

In two other patients the agglutination of the patient's cells in antiglobulin sera was relatively weak. Attempts to prepare more specific antiglobulin sera by injecting sera from these patients into rabbits were not successful.

Table 8

Prozone phenomenon, comparison of the agglutination of the cells of patient E H by antiglobulin serum upon immediate centrifugation and upon centrifugation after a period of incubation

Dilutions of antiglobulin serum	Cell agglutination	
	Immediate centrifugation	Incubation ½ hour
1-5	2	—
1 10	4	—
1-20	3	±
1 40	3	1
1 80	3	2
1 160	2	2
1 320	±	3
1-640	—	3
1 1280	—	1
1 2560	—	—

Variations in the antiglobulin serum reactions of other patients were observed that are of significance. The prozone phenomenon was encountered in the agglutination of the cells of one patient in varying dilutions of antiglobulin serum. Prozoning was eliminated by immediate centrifugation of the cell antiglobulin serum suspension (table 8) Agglutination of one patient's cells in antiglobulin serum was almost completely reversed in all dilutions by incubation of one-half hour at room temperature (table 9) The

same red blood cells were still agglutinable in 30 percent beef albumin, indicating that sensitization persisted. Normal red blood cells sensitized in varying dilutions of the patient's serum exhibited the same reversal of agglutination on incubation. These observations

Table 9

Prozone phenomenon, the effect of immediate centrifugation and centrifugation after incubation at room temperature on the agglutination of the cells of patient J. D. in dilutions of several antihuman globulin rabbit serum of approximately equal potency

Dilutions of antiglobulin serum	Cell agglutination					
	Immediate centrifugation			Incubation ½ hour		
	Serum #2	#3	#4	Serum #2	#3	#4
1:5	—	4	1	—	±	—
1:10	2	2	2	—	±	±
1:20	3	2	1	—	—	—
1:40	3	1	—	±	—	—
1:80	4	1	—	±	—	—
1:160	2	1	—	—	—	—
1:320	2	—	—	—	—	—
1:640	2	—	—	—	—	—
1:1280	1	—	—	—	—	—
1:2560	—	—	—	—	—	—

indicate that the reversibility of agglutination was due to a property of the sensitizing agent, possibly an enzymatic action on the rabbit globulin.

Association-dissociation equilibrium

The presence of unattached autoantibody in serum is probably dependent on the amount produced and the degree of saturation of receptors on the red blood cells. There is evidence that dissociation of autoantibody from the cell takes place continuously, indicating that an association-dissociation equilibrium exists. When sensitized cells are injected into the circulation of a normal individual the recipient's cells become agglutinable in antiglobulin serum, presumably due to a dissociation and transfer of autoantibody. It can be demonstrated *in vitro* that dissociation occurs continuously at 37 C without the destruction of the sensitized cells. When washed sensi

tized group A cells are mixed and incubated for several hours with normal group B cells, and then separated by means of anti A sera, the group B cells can be shown to have acquired sensitization by their agglutinability in antiglobulin serum. Since there was no evidence of hemolysis during incubation it is apparent that destruction of the cell is not necessary for dissociation and transfer of autoantibody

Cold agglutinins

Four patients exhibited high serum titers of bivalent cold agglutinins that sensitized red blood cells and produced agglutination below body temperature, but were eluted from the cell at 37°C. Red blood cells collected from these patients at body temperature and maintained at 37°C during washing showed the usual evidence of sensitization with the antiglobulin serum technique. The sera of two patients produced sensitization of normal cells by a univalent antibody at 5 C, which did not become dissociated at 37°C during washing of the cells. The sera of the other two patients presented only the bivalent saline cold agglutinin A nivalent antibody cold or warm, could not be demonstrated by the antiglobuli serum technique nor with the use of trypsinized cells.

Attempts to transfer the antibody found at body temperature from the cells of patients with cold agglutinins to normal cells were unsuccessful Furthermore, sensitization of normal cells did not occur with eluates prepared from the patient s cells by various other techniques. In contrast, eluates made from cells of patients without high titers f cold agglutinins have shown a high degree of activity usually greater at 37 C than at l we temperatures. The failure to obtain active antibody in eluates from the patients with cold agglutin ns p ints to qualitative difference in the nature of the antibody protein.

Limitations of serologic methods

A feature of autoimmune hemolytic disease requiring explanation is the persistence of red blood cell sensitization during remission f the disease, spontaneous or induced by cortisone or splenect my The persistance of autoantibody on the cell indicates that the mere adherence f the plasma protein to the surface is not sufficient to bring about cell destruction.

Our fundamental understanding of the mechanism of red blood cell destructio in autoimmune hemolytic disease is dependent on quantitati studies f free and adsorbed antibody concentrations.

In general, a reduction in concentration of adsorbed antibody accompanies cessation in abnormal red blood cell destruction.

Serologic methods of limited accuracy are the currently available tools for quantitative study. It is probable that critical changes in concentration of adsorbed antibody occur without being reflected in agglutinability in dilutions of antiglobulin serum (table 10). The activity of eluates prepared from samples agglutinating at 1:320 titer varies markedly.

Table 10

Serologic quantitation of adsorbed antibody

Dilution of Anti-D serum	Highest titer antiglobulin serum producing agglutination	Agglutination of D cells exposed to dilutions of eluate from sensitized cells, by antiglobulin serum				
		1:1	1:2	1:4	1:8	1:16
1:5	1:320	4	4	3	1	—
1:50	1:320	2	±	—		—

The results indicate that cells with identical agglutinability in high dilutions of antiglobulin serum may have varying quantities of adsorbed antibody as judged by the amount of antibody demonstrable in an eluate. 0.5 cc. of group O CDE cells were sensitized in 2 cc. volumes of Anti-D serum varying in concentration. After determining their agglutinability in antiglobulin serum dilutions, eluates were prepared by heating 0.5 cc. of the cells in 2 cc. of saline. Normal CDE cells were then exposed to serial dilutions of the eluate and the agglutinability tested by agglutination in 1:5 dilution of antiglobulin serum.

The relationships of antibody adsorbed on cell surfaces to the active and quiescent stages of autoimmune hemolytic disease are shown in tables 11, 12, 13. With spontaneous remission the agglutinability of the cells diminishes. Cortisone therapy suppressing the formation of autoantibody produces a reduction not only in free serum antibody but in agglutinability of red blood cells. Splenectomy in nine patients was followed by remissions of variable duration. Three of these patients did not manifest a significant change in the direct antiglobulin serum test even though splenectomy resulted in remission.

Idiopathic thrombocytopenic purpura

Most cases of idiopathic thrombocytopenic purpura are due to abnormality of plasma proteins similar to that seen in autoimmune hemolytic disease. More than one half the living infants born to

mothers with diopathic thrombocytopenic purpura show transient thrombocytopenic purpura. A platelet-depressing factor has been demonstrated in 16 of 26 patients with idiopathic thrombocytopenic purpura, and the frequency of demonstration of platelet agglutina tion has aried from 28 to 66 percent in the series studied to date.

One disadvantage in the study of serologic abnormalities in idiopathic thrombocytopenic purpura is the necessity of demonstrat

Table 11

Antibody concentration determined by direct antiglobulin serum dilution tests in autoimmune hemolytic disease during its active state and fter spontaneous remission

Pt.	Sex	Age	Active Disease		Remission		Remarks
			Low	High	Low	High	
J.D	M	52	1 160 1 160	1 1280 1 1280	0	1-80	Spontaneous re-mission follo ed by relapse
W.G.	M	31	1 160	1 320	1 1 1 160	1 10	Active hemolyses did not return
S G	M	42	1-50	1-640	1 10	1-640	Auto immune hemolytic dis-ease accompany ing viral pneumonia
H.K.	M	42	1 200	1-400	1 10	1-50	Auto-immune hemolytic dis-ease accompany ing viral pneumonia

ing the immune agent in plasma. Free serum antibody may exist in low concentrations in some patients, as is the case in many with aut immune hemolytic disease. Because of the difficulty in obtain ing stable platelet suspension after the washing required, the use of the direct antiglobulin serum test was not successful. Although patients with acute and chronic thrombocytopenic purpu a rapidly rem ve transfused platelets from the circulation, the disappearance of transfused platelets in idiopathic thrombocytopenic purpura needs re-evaluation. It has been postulated that isoantibodies formed as the result of previous transfusions may reduce the survival time

of platelets. Platelet agglutinins, like red blood cell autoantibodies, are not always panagglutinins, since the serum of one patient may agglutinate aut logous platelets, but not agglutinate all other platelets tested.

Table 12

Antibody concentration determined by direct antiglobulin serum tests in autoimmune hemolytic disease during its active state and after emission following cortisone therapy

Pt.	Sex	Age	Active Disease		Remission		Remarks
			Low	High	Low	High	
D.H.	F	14	1 400	1-400	0	1-200	Complete remission with negative direct antiglobulin test
L.P	M	32	1 320 1 160	1-640 1 1280	1-5	1-40	Quiescent state relapse with reduction f cortisone
H.B.	F	65	1-400 1 200	1 800	1-50	1-50	Remission with relapse following withdrawal
L M	M	52	1-640	1-640	1 160	1 160	Free serum antibody disappeared during partial remission
W H.	M	47	1 3200	1 6400	1-400	1 1600	Partial remission prior to splenectomy
(S	F	76	1 6400	1 12800	1 200	1-400	Partial remission prior to splenectomy

Neutropenia and lupus

The probab lity that some instances f unexplained neutropenia, particularly those respo ding to plenect my are due to autoantibodies still rests largely on the circumstanti l evidence f association.

Evidence is accumulating that facto in the serum f patients with disseminated lupus erythematosis, responsible f the lupus

cell and for clumping of leucocytes in L. E. preparations, belongs to the category of abnormalities of plasma proteins referred to as autoantibodies. Other studies have shown that antileucocytes produce changes in leucocytes making them indistinguishable from

Table 13

Antibody concentrations determined by direct antiglobulin serum dilution tests in autoimmune hemolytic disease during its active state and after remission following splenectomy

Pt.	Sex	Age	Active Disease		Remission		Remark
			Low	High	Low	High	
G.T	F	64	1 160	1-320	1 10	1 160	Remission
J.Z.	F	56	1 160	1-640	1 10	1-80	Remission
L.P	M	82	1 160	1 1280	1-40	1-640	Remission
O P	M	32	1-80	1 160	1-20	1 160	Transient relapse following splenectomy
F.H.	M	58	1 160 1-320	1 640 1-640	1-5	1-80	Relapse 3 mos. after steroid spleen removed
J.D	M	52	1 160 1-320	1 1280 1-320	1 20	1 320	Remission with relapse after 1 year
W.H.	M	87	1-800	1-3200	1-400	1 1600	Remission 2 yrs.
H R.	F	49	1 320	1 640	1 320	1-640	ACTH given following splenectomy
L.T	F	67	1-800	1-6400	1-800	1 1600	Remission 3 mos.

l pus cells. Agglutinins for platelets, an autoantibody for red blood cells, and a fact producing the lupus cell have all been demonstrated in patient with lupus erythematosus with hemolytic anemia and thrombocyt penic purpura. The protein abnormalities respon sible for these phenomena appeared to be separate and distinct, since the red cell-sensitizing agent eluted from preparations of red cell stroma, while acti e in sensitization of normal red cells, did not produce agglutination of platelets or the l pus cell changes in leucocytes.

The Metabolism of Mucopolysaccharides

DR. ALBERT DORFMAN In hypersensitivity states and related diseases, profound alterations occur in the ground substance of connective tissue. The chemical and physicochemical nature of these changes is almost entirely unknown despite widespread speculations based on histochemical studies.

Ground substance contains at least two acid mucopolysaccharides, hyaluronic acid and chondroitin sulfuric acid. The known chemical and physicochemical properties of these polyelectrolytes suggest that they may play an important part in the physiologic and pathologic behavior of connective tissue although the lack of specific data regarding their biochemistry makes an analysis of their functional potentialities impossible.

Isolation of hyaluronic acid

These studies have been carried out in collaboration with Roseman. As an experimental tool, Group A hemolytic streptococci offered an unusual opportunity for the study of the biosynthesis of hyaluronic acid, since biochemical experience has indicated that the pathways of metabolism in microorganisms bear close resemblance to those of mammalian tissue. The unique presence of hyaluronic acid in the capsule of the streptococcus, which is apparently chemically similar if not identical to that of connective tissue, is of particular interest in view of the peculiar character of the reactions of the human to streptococcal infection.

A strain of Group A hemolytic streptococcus was cultured in a semisynthetic medium, and methods were devised for the isolation of hyaluronic acid from this culture. The high degree of purity of this preparation was established by both multiple analyses and electrophoresis. In the analytical results shown in table 14, the somewhat low values for uronic acid, as evidenced both by uronic CO_2 analysis and neutralization equivalent, are apparent. Similar discrepancies are present in the original data of Kendall and Heidelberger and may be of some significance in indicating chemical differences between streptococcal and connective tissue hyaluronic acid. If such differences exist they may open up new possibilities with respect to the effects of streptococcal products on mammalian tissues.

The hyaluronic acid isolated from the culture supernatant was subjected to degradation procedures which made possible the de

termination of the location of radioactive carbon in the various parts of the molecule.

Table 14

Analysis of hyaluronic acid

Analysis	Method	Observed	Theory	Observed / Theory
		percent	percent	
C	Microcombustion	44.37	44.33	1.00
H		5.93	5.58	1.06
N	Kjeldahl Nessler	3.7?	3.69	1.01
	Dumas	3.55		.96
Uronic CO_2	Gasometric	10.31	11.60	.89
	Titrimetric	10.60		.91
N Acetyl	Chromic Acid	11.74	11.35	1.03
Neutralization equivalent	Titrimetric	355	379	.94
Ash S P		—		
Glycogen	I₂	—		
Hyaluronidase	Turbidimetric	+++		
[α]		−67.5		

All analyses performed on dried samples or corrected for moisture content
**Based upon $(C_{14}H_{20}O_{11}N)$

Hyaluronic acid precursors

To establish the precursors of hyaluronic acid, various compounds, into which radioactive carbon had been introduced in specific positions, were incorporated into a semisynthetic medium upon which a strain of streptococcus was grown. When 1-C^{14}-glucose was incorporated into the growth medium with a radioactivity of 30,858 c.p.m. (measured as $BaCO_3$ at infinite thickness) pure hy

aluronic acid was isolated with an average radioactivity of the 14 carbon atoms f hyaluronic acid f 5,231 c.p.m.

After hydrolysis of the polysaccharide, glucosamine, acetic acid, and carbon di xide produced by the decarboxylation f uronic acid were isolated. A portion of the glucosamine was oxidized t glucosaminic acid, and the latter decarboxylated with ninhydrin to yield carbon dioxide, which represents the first carbon atom of the glucosamine. From the residue f the decarboxylation reaction rabinose was isolated, giving a measure f the radioactivity of the C_{2-6} f the glucosamine molecule. In addition to these components *acetic and lactic cids were isolated f om the medium as benzimidazole derivatives.*

The radioactivity f the first carbon atom f the glucosamine was very similar to that of the glucose in the medium, indicating that the glucosamine portion of the hyaluronic acid molecule rises from the glucose with ut previous scission f the glucose m lecule. The radioactivity of the acetyl porti n f the m lecule was found t be identical with that f the acetate f the medium, suggesting that the two either rise from the same precursors o are in equilibrium. The radioactivity of the sixth carbon atom f the uronic acid was found to be l w as was that f C_{2-6} f the glucosamine, i dicating that under the conditions of these experiments little resynthesis f glucose from glycolytic products occurs. When the total amount f radioactivity of the hyaluronic acid was compared with that accounted fo by the parts that were isolated and degraded, there was a discrepancy f 29,678 counts. Since only the first fi e carbon atoms of the uronic acid were ot isolated, it f l l ws that this amount of radioactivity must reside in these carbon atoms. This could be readily accounted f by activity in the first carbon atom comparable t that f the first carbon atom of the original glucose, but was not established with certainty in this experiment. Because the isolation and degradation f the uronic acid portion f the molecule presents considerable experimental difficulty a simpler method was sought. Accordingly 6-C^{14}-glucose was synthesized and incorporated into the medium. The radioactivity f the sixth carbon atom i the uronic acid (which is readily obtained by decarboxylation) was found comparable to that of *the original glucose suggesting that the uronic acid* portion of the hyaluronic acid is formed from glucose without previous scission f the molecule. The C_6 of the glucosamine was isolated and showed comparabl radioactivity f rnishing confirmation regarding the rigin of the hexosamine.

With the remote origin of the fourteen carbon atoms of hyaluronic acid established, recent experiments have been aimed at the elucidation of the more immediate precursors. Utilizing glucosamine labeled with C^{14} in the one position and N^{15} in the amino group we sought to determine whether glucosamine as such is incorporated into the polysaccharides. The doubly labeled glucosamine was prepared biologically by growing *Aspergillus niger* in the presence of 1-C^{14}-glucose and $N^{15}H_4NO_3$. Labeled glucosamine was isolated from the chitin of the mycelium and incorporated into the medium for streptococci. The remainder of the experiment was then conducted as previously discussed. The finding of a constant C^{14}/N^{15} ratio indicates that glucosamine is used directly in hyaluronic acid synthesis.

To determine whether glucuronic acid was incorporated as such, 6-C^{14}-glucuronolactone was synthesized and utilized as an intermediate. The isolation of the sixth carbon atom of the glucuronic acid portion of the hyaluronic acid indicated no incorporation of the glucuronolactone. Since the lack of incorporation of glucuronic acid might be due to its glycolysis prior to hyaluronic acid synthesis, other organic acids were isolated from the medium. These showed no radioactivity suggesting that glucuronic acid is not metabolized *via* this pathway. This failure to utilize uronic acid may be due to impermeability of the cell, or may indicate that oxidation of the sixth carbon atom occurs only after glycoside formation.

In subsequent studies utilizing carboxyl-labeled acetic acid and carboxyl-labeled N-acetyl glucosamine we have demonstrated that acetic acid can serve as a precursor of the N-acetyl group of hyaluronic acid, and that a deacetylase exists that can hydrolyze the acetyl group of N-acetyl glucosamine. The evidence obtained thus far suggests that N-acetylglucosamine is not utilized for hyaluronic acid synthesis.

Mucopolysaccharide metabolism in skin

After development of a method for the isolation of hyaluronic acid and chondroitin sulfuric acid from skin, a study of mucopolysaccharide metabolism in mammalian skin was instituted. These studies have been carried out in collaboration with Schiller. Defatted ground skin was extracted with a 0.2 percent solution of sodium hydroxide. Following dialysis and adjustment of pH the extract was treated with trypsin and further dialyzed. Proteins were precipitated with trichloracetic acid and the polysaccharide precipitated

from the supernatant with alcohol. The crude mixture of poly saccharides so isolated was separated by zone electrophoresis on celite.

Two tenths of a millicurie of carboxyl labeled acetate was given to rabbits subcutaneously daily for periods up to eight days. Following this the animals were sacrificed and polysaccharides were isolated from the skin. A greater rate of synthesis of hyaluronic acid than chondroitinsulfuric acid was apparent. These same results were obtained whenever 1-C^{14}-glucose was administered.

Subsequent experiments studying the rate of disappearance of hyaluronic acid and chondroitinsulfuric acid from skin have confirmed these conclusions. Hyaluronic acid has a half life time of between two and three days in rabbit skin while chondroitin has a half life time of about eight days. Experiments utilizing C labeled acetate C labeled glucose, and $S^{35}O_4$ suggest that the rate measured represents complete breakdown and resynthesis of the poly saccharide molecule.

Hyaluronidase Inhibitor and Heparin in Human Blood Serum

DR. DAVID GLICK: Studies on the nature and role of the nonspecific hyaluronidase inhibitor existing in human blood serum, possibly employed as a defensive agent by higher animals against hyaluronidase, have been in progress for several years. It has been established that higher forms of life possess at least two mechanisms for the inhibition of the hyaluronidase elaborated by an invading organism. The integrity of defensive intercellular walls may depend on these mechanisms.

The production of specific antibody in response to a specific enzyme elaborated by an invading species constitutes one of these mechanisms. Since this method of defense involves a time factor for production of antibody, a general and more immediate available defense factor would seem desirable to inhibit the activity of hyaluronidase from any source.

Heparin and hyaluronidase inhibitor

A relationship between heparin and such a nonspecific hyaluronidase inhibitor is suggested from findings that the serum concentrations of both are elevated during periods of stress, such as in

bition usually tends to be of the all or none variety. For quantitative studies it is necessary to use connective tissue membranes *in vitro* such as rabbit urinary bladders, or intact membranes *in vivo*. For the latter the *talocrural articulation of the rabbit was selected.* This joint, consisting of a small compartment lined with a thin membrane, is less complex than the knee joint.

Table 15

The influence of various substances on the permeability of the ground substance

Enhancement	Inhibition
Hyaluronidases	Cortisone
Lysozyme	Adrenal Cortical Extract
Desoxyribonuclease	Estrone
Ascorbic A id	Testosterone
Dehydro-ascorbic Acid Spreading facto *from human urine*	Salicylates

*Determined by the spreading reaction

While the spreading reaction is independent of local circula t ry phenomena, *in vivo* methods that involve clearances may not be. The qualitative results, h wever are identical with those obtained with isolated membranes with preading tests.

Synovial membrane permeability

The anio ic dyestuff PSP (phenolsulfophthalein) injected into the talocrural articulatio of the rabbit is rapidly absorbed and excreted in the urine. This pattern of rapid clearance of PSP is altered by ariety of factors. *Hyaluronidase injected* int the synovial cavity along with the dye produces an increase in the rate of dyestuff elimination. The same rapid disappearance of PSP from the synovial cavity is observed in nephrectomized rabbits. H wever th injection of hyaluronidase into the ynovial cavity does not increase the clearance of intravenously administered PSP

Intramuscular desoxycorticosterone (1 mg per kg. of body

weight) administered before the dye is injected, acts similarly to hyaluronidase. These drugs together do not produce an enhanced effect. An increase in permeability is bserved after the intra articular injection of lysozyme or desoxyribonuclease, or massage f the joint.

Cortisone or ACTH dministered before the dyestuff completely inhibits synovial membrane permeability Hyaluronidase does not antagonize the cortisone effect. Estrone, testosterone, and ascorbic acid decrease the permeability to a lesser degree than does cortisone.

Adrenalectomized rabbits show an inhibition f the normal spreading reaction. Hyaluronidase enhances this reaction pro-portion t the amount f enzyme administered, and is independent of the presence r absence f adrenal glands. Adrenalectomy in rabbits decreases the permeability f the synovial membrane in vivo Hyal ronidase is effective in increasing the membrane perme ability f drenalectomized rabbits. Cortisone completely block membrane permeability whil ACTH is without effect i the dren-alectomized animal.

These data indicate that the permeability f the ground sub-stance is markedly influenced by the presence f the adrenals, and most agents which depress permeability do so through an adrenal mechanism. Few substances have a direct effect.

The accumulation of hyaluronic acid in some tissues during thyroid dysfunction and the antipermeability effect of estron and testosterone indicate that the ground substance is affected by the thyroid and gonads as well as by the adrenals.

Since these studies demonstrate that the ground substance is markedly influenced by sever l physiologically occurring sub-stances, it is ot necessary to invoke the presence f hyaluronidase in tissues as a normal physi logic agent. Although it is not possible to demonstrate hyaluronidase in tissues outside the sperm, current assay methods may not detect concentrations of the enzyme that might be active elsewhere.

Permeability in experimental serum disease

T determine whether the beneficial effects f ad enal stimula-tion in nephrotoxic serum disease in the rabbit is due to suppression *f lymphoid tissue alteration f permeability f ground substance* t antigen r ntibody comparison was made of the effects f cortisone, 21 acetoxypregnenol ne, desoxycorticosterone and hy-l ronidase in experimental serum disease.

Cortisone affects the ground substance as well as lymphoid

tissue while 21-acetoxypregnenolone stimulates the adrenals to release an antipermeability factor and has little effect on lymphoid tissu Hyaluronidase enhances the permeability only of the ground substance. Cortisone was found to prevent the cardiovascular les ns f nephrotoxic serum disease. This protection could be due only in part to antipermeability action because 21 acetoxypregnenolone given in dose calculated to equal the antipermeability effect of cortisone was without influence on the lesions.

Hyaluronidase markedly increases the incidence and severity of lesions in the heart and blood vessels. These observations indicate the pronounced influence of permeability in the course of a sensi tivity disease.

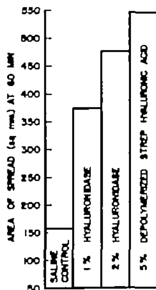


Figure 14 Activity f depolymerized hyaluronic acid as spreading factor relative t hyaluronidase.

Peptizing property of hyaluronate

The subcutaneous injectio of rabbits with hyaluronidase results in an increase of hyaluronate-like staining material in the distal tubule and a discharge of hyaluronate int the tubula lumen The subcutaneous injecti n of hyaluronidase in patients with renal calculi excreting turbid urine is f llowed by the excretion of clear urine. Utilizing *in vitro* methods it can be demonstrated that hy aluronate is a peptizing agent and protecti colloid.

If the effect of hy luronidase on urine is the result f release and activ tion of hyaluronic acid, then stim lati n f the adrenals

or the injection of cortisone should have the reverse effect. Subjecting a healthy adult to stress, injection of cortisone, or injection of ACTH leads to the production of turbid urine which is not due to phosphaturia or change in urinary pH. The urinary excretion of glucuronides, an indirect measure of hyaluronic acid, is decreased.

The fact that hyaluronic acid present in the circulation finds its way into the urine suggests the possibility that hyaluronic acid itself may be an active spreading factor if present in the proper molecular form. Depolymerized streptococcal hyaluronic acid has been found to be as active spreading factor as hyaluronidase (figure 14).

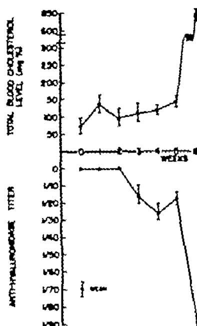


Figure 15. Relationship between serum cholesterol and antihyaluronidase titer in rabbits fed high cholesterol diet and injected with hyaluronidase.

Hyaluronidase in hypercholesterolemia

Hypercholesterolemia experimentally produced in rabbits may be controlled for a limited time by the subcutaneous administration of hyaluronidase. Concomitant with the production of an antihyaluronidase, an abrupt increase in blood cholesterol is observed (figure 15). The period of protection afforded by hyaluronidase does not spare the rabbits from the pathologic changes associated

with hypercholesterolemia. Greater uptake of cholesterol by endothelial cells was apparent in the treated rabbits, which was consistent with the higher degree of dispersion of serum lipids noted on dark field microscopy Endogenous hypercholesterolemia as seen in rats with nephrotoxic serum disease is also controlled by the administration of hyaluronidase or hyaluronic acid

The administration of large doses of hyaluronic acid, isolated from a highly virulent strain of streptococcus, in acute toxicity studies resulted in a mortality of one in 250 animals there were no histopathologic changes in the tissues of this animal. The pathologic manifestations observed during infections with this type of organism are therefore not likely due to the hyaluronic acid such organisms might release.

Much remains to be learned of the normal physi logy of the ground substance, the changes that occur in it during pathologic states, and the way in which drugs achieve therapeutic results in disease.

Discussion

CHAIRMAN ARTHUR L. BLOOMFIELD In light of what was said regarding the inhibition f the immune reaction by steroid, would you say such therapy should be discontinued at the first sympt m of infection, viral or bacterial, or is it too late then?

DR. RAFFEL Answering this from the experimental tandpoi t, such therapy should be discontinued for reasons f infection I don t think that the allergic reactivity which accompanies most infect ous processes is such as to do obvious early inj ry to the host f om the sta dpoi t purely of allergic destructiveness hence there is no good basis for providing ster ids simply t depress this phase of the host response And ince these substances seem t encourage the infectious process it would seem to be a good idea to discontinue them

CHAIRMAN BLOOMFIELD Since decrease in eosinophiles foll w dministration of ACTH r cortisone, is there any evidence that they re the carriers of either antigen or antibody?

DR. DOUGHERTY The best evidence at presence is that i travenous cortical hormone does ot increase sequestration f eosinophiles in the spleen, bone marrow or lungs. Thus eosin penia is ot due to a shunting ut f eosinophiles into these areas. The eosinopenia is most likely due to lytic action of the hormone which produces a degranulation f the cell that is very important from clinical standpoint for those who use the eosinophile count, since the umber of granules that remai determine whether you

classify the cell and eosinophile or not. This is probably one of the reasons for the discrepancies found in eosinophile counts. There is work suggesting that antigen is contained in eosin philes. Guinea pig eosinophiles given passively to other guinea pigs immunized to the homologous antigen may shock. This can be prevented by heparin.

CHAIRMAN BLOOMFIELD Is there any evidence that steroid therapy either inhibits or accelerates the formation of histamine from the antigen-antibody complex?

DR. GOOD The only evidence I know about is the evidence that antibody complexes cannot inhibit a histamine reaction.

CHAIRMAN BLOOMFIELD Are we any closer to understanding the role of the thymus gland?

DR. THOMAS No.

CHAIRMAN BLOOMFIELD Can concentrated human gamma globulin be in any way considered nonspecific?

DR. THOMAS Yes.

CHAIRMAN BLOOMFIELD Why does the passive immunity f the newborn to rubeola and poliomyelitis last for six to nine months? Is it purely a quantitative matter?

DR. DIXON I think it probably is. The duration of passive immunity would be determined by the rate of catabolism of antibody in early childhood. Antibody decreasing with a half life of three or four weeks might well cover the infant f r that period of time

DR. DORFMAN There is no simple chemical mechanism or reasonable metabolic pathways by which a mucoprotein can arise through destruction f mucopolysaccharide. Thus, relating a rise in serum mucoprotein to change in connective tissue mucopoly saccharide is without reasonable basis.

There are many theoretical reasons why one should be dubious about the role of hyaluronidase and hyaluronidase inhibitors in such disease states as rheumatic fever Hyaluronidase may play no more role in the breakd wn of hyaluronic acid than does amylase in glycogen degradation, synthesis and storage. The biochemistry of ground substance is extremely complex and we know little bout it at present.

DR. EHRICH: If hyaluronidase inhibitor is heparin, its action in preventing fibrinoid and fibrin f rmation in disease may well be nterpreted in teleological sense in the natural defense mechanism.

DR. GLICK Teleology has its uses primarily in serving as guide. It can give direction to experimental design. It also has it weaknesses and is not t be accepted uncritically T leology has

been compared to a woman with whom you like to keep company but with whom you don't want to be seen on the street.

I don't think it is entirely accidental that nature has endowed many predators with enzyme hyaluronidase. Many pathogenic or ganisms possess this enzyme and in nature every object of prey has some kind of defense to the predator It is not unreaso able to assume that hyaluronidase inhibitor plays a defensive role against the invasive factor but this is offered as a possibility not as a fact.

CHAIRMAN BLOOMFIELD A clinician who sees rheumatic fever might ask this question. You have first a hemolytic streptococcus infection of the throat, tonsillitis, which subsides. There is no more hyaluronidase getting into the blood stream after the cute phase and still there is a latent period of three weeks longer befo e the onset of rheumatic fever How do you fit hyaluronidase into this?

DR. GLICK My guess would be that the invading rganism is highly adaptive. It has been shown by *in vitro* experiments that organisms grown in a hyaluronic acid-rich medium increase their hyaluronidase. The latent period might involve a development of the enzyme to increase invasiveness of the streptococcus to critical level.

DR. EHRICH There may be simpler explanation. The streptococci that produce rheumatic fever are those which f rm capsule f hyaluronic acid. This capsule interferes with phagocytosis. Hence, the bacteria will liv longer in tissues and thus elicit more anti body in the host. It has been noted that those patients developing rheumati fever are those that produce higher antibody titers than others. I think it is the hyaluronic acid nature f the capsul rathe than the hyaluronidase which determine the end result. The two types of streptococci which are the best producers of hyaluronidase (Types 4 and 22) are never observed in connection with rheumatic fever

DR. DORFMAN The properties f hyaluronidase inhibit are chemically very different from heparin. If heparin plays a ole it can only be as part of a complex, and the direct presence of heparin and p re hyaluronidase inhibitor has not been dem nstrated. We have recently been able to isolate the blood hyal ronidase inhibit in p re form and have been unabl to find evidence of the presence of heparin.

DR. DOWNIE In regard to the question of persistence of anti body formation in the absence of antigen it may be very difficult to be sure that there is antigen present. I agree with Dr Dixon that there is a good deal f evidence to suggest that antibody formation

may go on in the absence of antigen after it has been eliminated from the tissue. If plasma cells are concerned with antibody formation I should like to ask what is the life span of plasma cells, and if they live a short time do they divide to other cells which continue to form antibody? Does antigen influence the cell so that the property of forming antibody may be passed on to these cells?